Functional Genomics of Ageing and Its Modulation by Diet

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

by

Daniel A. Wuttke

March 2017

Abstract

Ageing is a widespread phenomenon limiting the lifespan of almost all species. Virtually all organisms age, however what controls the ageing process remains enigmatic.

A lot of human disease and death are affected by ageing or age-related diseases. Understanding the ageing process is crucial to benefit humanity including intervention of human disease or life extension. The work presented here allows to gain insights into the ageing process which can be manipulated by targeting ageing genes via genetic modifications, environmental factors, or pharmacological interventions.

Biological information is increasing at an exponential pace. Information technology is also advancing at a fast pace. The combination of these two trends may allow us to understand biological processes such as ageing.

Ageing is plastic; many studies have shown that the ageing process can be sped up, slowed down, stopped, or even reversed. For example, the ageing process can be slowed down by dietary restriction (DR), the most powerful non-genetic intervention known to counteract the basic ageing process. In addition, the ageing process can be modulated by genetic as well as environmental means including dietary as well as pharmacological interventions. Here it is hypothesized that ageing genes do not only play an important role in the ageing process but also in its modulation by diet.

In this thesis, functional genomics approaches are applied to genomic data such as transcriptomics and interactomics. Graph theoretic concepts like guilt-by-association and shortest path are applied on ageing and its dietary manipulations. This methodology enabled to gather new insights into the underlying mechanisms of the ageing process and its interference with interventions like dietary restriction, as well as to predict original hypotheses, new ageing genes, and novel pharmaceuticals.

Firstly, a curated knowledge base was established which includes loss- and gain-of-function experiments that impact on lifespan of organisms including human, mouse, fruit fly, nematode, and yeast, among others. Genes controlling the ageing process (ageing genes) were classified based on their effect on lifespan resulting from genetic manipulations in the common biomedical model organisms as well as in humans. Those classes were characterized for common associations and potential novel ageing genes were predicted by applying graph theory like the guilt-by-association principle. Specifically, this thesis classifies ageing genes that control the ageing process into two main classes: 1) gerontogenes that mediate the ageing process, therefore decrease lifespan of the organism; 2) ageing-suppressor genes that suppress the ageing process, thus increase lifespan of the organism. Further analysis shows that:

Gerontogenes are associated with translation, positive regulation of apoptosis, inflammation, TOR signalling, positive regulation of multicellular organism growth and development, as well as transcription regulation.

In contrast, ageing-suppressor genes are associated with DNA metabolism (including DNA repair, telomere maintenance, and chromatin organization), stress response, negative regulation of apoptosis, protein homeostasis (including protein repair, regulation of protein stability, lysosome/autophagy, and the ubiquitin-proteasome system), and endocytosis, as well as transcriptional regulation.

Thus, gerontogenes were found to be involved in growth/development and translation, while ageing-suppressor genes are involved in stress response and diverse repair processes.

Secondly, a class of genes, denoted here as dietary restriction-essential genes (DR genes for short), which are necessary for the lifespan-extending effect of dietary restriction in commonly used biomedical models organisms was found. DR genes were defined and characterized as well as new candidate genes predicted. It was found that DR genes are evolutionary conserved on the molecular (sequence) level more than expected by chance (even more than ageing genes which are conserved) and are more likely to interact with each other based on molecular interactions than expected by chance. The predicted genes were experimentally validated and prediction accuracy was found to be up to 89%. The new DR genes, which were predicted by guilt-by-association, include *FRE6*, *RCR2*, and *OPT2*. *FRE6* and *OPT2* were found to be induced on the transcriptional level upon DR, while *RCR2* found to be transcriptionally repressed. *OPT2* was one of the greatest upregulated genes upon DR. Potential transcription factors controlling the DR response were identified.

Thirdly, tissue-specific and common molecular signatures of gene activity changes during ageing and dietary restriction for different organisms (human and common biomedical models) were generated. Those signatures were characterized for their associations and the associations common to multiple species identified. Ageing commonly upregulates inflammation, MAPK and TOR signalling, and downregulates proteostasis, cell cycle, and epigenetic modifications. Accelerated ageing commonly upregulates inflammation as well as Notch signalling and downregulates Wnt signalling. Cellular senescence commonly upregulates inflammation and negatively regulates cell cycle and apoptosis. DR commonly upregulates specific gene regulation and nuclear processes including chromatin silencing while downregulating MAPK signalling and other transcriptional regulation. DR in particular modulates stress response, circadian clock, and proteostasis.

Lastly, via graph theory and pattern matching, drugs that target specific classes of ageing genes as well as potentially reverse ageing-related gene expression changes or mimic the effect of dietary restriction on the level of the transcriptome were predicted. Drug mesalazine, among others, was predicted as the top compound to transcriptionally mimic the effect of dietary restriction while others like LY-294002 seem also capable of additionally reversing ageing, accelerated ageing and cellular senescence. Among the top drugs and supplements were well-known anti-ageing compounds as well dietary restriction mimetics validating the approach.

The ageing process and its modulation by diet appear to be governed by specific gene classes, that are conserved and interact with each other. These features can be used to identify lifespan extending processes, functions, components, and entities via the utilization of different omics like interactomics and transcriptomics that allow to investigate the interactions and activities of genes. Gerontogenes by their very activity drive processes that mediate ageing, while ageing-suppressor genes control processes that counteract ageing. DR genes mediate the effect of a restricted diet to modulate those activities in order to slow down ageing. Small molecules were identified that potentially target specific ageing gene classes, reverse ageing, Hutchinson-Gilford progeria syndrome, and cellular senescence, or mimic the effect of DR and may therefore enable to intervene with the ageing process pharmacologically.

Declaration

I hereby clarify that this dissertation constitutes my own product, that where the language of other is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expression or writings of another.

I declare that the dissertation describes original work that has not yet previously been presented for the award of any other degree of any institution.

I hereby confirm that all the computational work in this thesis has been done by myself. Experimental validation was performed by Dr. Fusheng Tang.

This dissertation contains material that is confidential and/or commercial sensitive. It is included here on the understanding that this will not be revealed to any person not involved in the assessment process.

Signed,
Daniel Wuttke

Acknowledgements

I would like to thank João Pedro de Magalhães and Brian Merry for their support, patience and inspiration to make this work possible. I also like to thank Alex Freitas and Francesco Falciani for their extensive feedback and discussion on the thesis. Further, I like to express great thankfulness to Fusheng Tang for his passionate and extremely useful discussions/suggestions and effort for validating predicated candidates experimentally.

Contents

List of Figures

List of Tables

Glossary

Abeta
Amyloid Beta

AGE
Advanced Glycation Endproducts

AHR
Aryl Hydrocarbon Receptor

AL
ad libitum

AMD
Age-Related Macular Degeneration

ARVC
Right Ventricular Cardiomyopathy

ASLI
Asymmetric Spatial Learning Impairment

BER
Base Excision Repair

BMI
Body Mass Index

CALERIE
Comprehensive Assessment of the Long-term Effects of Reducing INtake of Energy

CD8
Cluster of Differentiation 8

CDT
Development Transcriptome Database

CMA
Chaperone-Mediated Autophagy

CNS
Central Nervous System

CQ
Coenzyme Q(10)

CR
Calorie/Caloric Restriction

DAVID
Database for Annotation, Visualization and Integrated Discovery

DHEA
Dehydroepiandrosterone

DIC
Differential Interference Contrast

DIC
Disseminated Intravascular Coagulation

DNA
Deoxyribonucleic Acid

DR
Dietary Restriction

EC
Entorhinal Cortex

EE
Early Endosome

EGF
Epidermal Growth Factor

FDR
False Discovery Rate

FTP
File Transfer Protocol

FUdR
5-Fluorodeoxyuridine or Floxuridine for short

GEO
Gene Expression Omnibus

GH
Growth Hormone

GO
Gene Ontology

GSH
Glutathione

GSSG
Glutathione Disulfide

GTE
Genotype-Tissue Expression

GWAS
Genome-Wide Association Study

GWLS
Genome-Wide Linkage Study

HC
Hippocampus

HCF
Host Cell Factor

HDM
Histone Demethylase

HGPS
Hutchinson-Gilford Progeria Syndrome

IGF
Insulin-Like Growth Factor

IIS
Insulin/IGF-I Signalling

ITP
Intervention Testing Program

KO
Knockout

LA
Lipoic Acid

LCD
Low Calorie Diet

LDA
Linear Discriminant Analysis

LE
Late Endosome

LH
Luteinizing Hormone

LPO
Lipid Peroxide

MALDI
Matrix-Assisted Laser Desorption/Ionization

MIF
Migration Inhibition Factor

MMTP
Major Mouse Testing Program

MR
Methionine restriction

MS
Mass Spectrometry

MSE
Middle-Sporulation Element

MVB
Multivesicular Body

MeSH
Medical Subject Heading

NCBI
National Center for Biotechnology Information

NIA
National Institute of Aging

PCG
Postcentral Gyrus

PCR
Polymerase Chain Reaction

PFC
Prefrontal Cortex

PI
Phosphoinositide

PKMT
Protein Lysine Methyltransferase

PLZF
Promyelocytic Leukaemia Zinc Finger

QTL
Quantitative Trait Loci

RA
Retonic acid

RAGE
Receptor for Advanced Glycation Endproduct

RNA-seq
RNA Sequencing

RNAi
RNA interference

RPE
Retinal Pigment Epithelium

SAGE
Serial Analysis of Gene Expression

SAH
S-Adenosyl-L-homocysteine

SAHF
Senescence-Associated Heterochromatin Foci

SAM
Serial Analysis of Microarray

SAM
S-Adenosyl Methionine

SAM
Significance Analysis of Microarray

SASP
Senescence-Associated Secretory Phenotype

SFG
Superior Frontal Gyrus

SG
Superior Gyrus

SGD
Saccharomyces Genome Database

SNP
Single-Nucleotide polymorphisms

SRA
Sequence Read Archive

SSC
Spermatogonial Stem Cell

STRE
Stress-Response Element

SVZ
Subventricular Zone

TFBS
Transcription Factor Binding Site

TOF
Time of Flight

TOR
Target of Rapamycin

TORC1
Target of Rapamycin Complex 1

TRITC
Tetramethylrhodamine

TSA
Trichostatin A

TSS
Transcription Start Site

UPR
Unfolded Protein Response

UPS
Ubiquitin Proteasome System

URS1
Upstream Regulatory Sequence 1

USD
United States Dollar
VC
Visual Cortex
WGA
Whole Genome Association
WGAS
Whole Genome Association Study
YEPD
Yeast Extract Peptone Dextrose
Y2H
Yeast Two-Hybrid
dN/dS
Non-synonymous Nucleotide Mutations Rate / Synonymous Nucleotide Mutations Rate
siRNA
Small interfering RNA

1 Introduction

1.1 Ageing

1.1.1 Definition of Ageing

Ageing is one of the oldest biological enigmas and a major biomedical problem of the 21st century. Ageing is an almost ubiquitous though not universal phenomenon in nature. It has been a research puzzle for decades as to why lifespan is limited and evolution has not eliminated this phenomenon. To understand ageing it is important to define it as precisely as our current understanding will allow. However, a consensus on a simple definition of ageing has not been achieved, evidence of the lack of an understanding of the mechanisms underlying the ageing process (Li, et al., 2014).

Increase in Probability of Death. Ageing is an increased liability to die, or an increasing loss of vigour, with increasing chronological age, or with the passage of life cycle (Comfort, 1960). Ageing is accompanied by deteriorative changes with time during postmaturational life that underlie an increasing vulnerability to challenges, thereby decreasing the ability of the organism to survive (Masoro, 1995). The ageing process renders individuals more susceptible as they grow older to the various factors, intrinsic or extrinsic, which may cause death (Maynard, 1962). Ageing can be defined as the increase in the probability of death within a period of time. In humans, from 0 to 9 years there is a decrease in the probability to die in the following year. After age nine the age-specific mortality rate increases and there is an exponential increase in this rate from around 30 years (Arking, 2006). Ageing therefore seems to be a natural phase in many organism's life cycle.

Natural Deterioration. Ageing is the observed natural deterioration of bodily functions, organs, and organ systems, thereby increasing the onset of disease and pathology, with a resulting loss of function eventually leading to death.

Functional Decline. By teleological criteria, development can be viewed as consisting of early processes that enhance the functional capacity of a system, whereas ageing consists of later processes that diminish or have no effects on ability to function (Kohn, 1978). Ageing is the progressive decline in several but not all physiological functions, resulting ultimately in the thermodynamic equilibrium, i.e. death. Ageing decreases most aspects (functionalities), although some kinds of cancer are less severe in elderly people as a result of diminishing rates of mitosis. Therefore defining ageing simply as functional decline is not sufficient.

Passing of Time. Ageing is generally used to describe a host of time-related alterations in biological entities from molecules to ecosystems (Finch, 1990). Ageing is a progressive, i.e. a time-dependent phenomenon. Ageing is (for a normally developing organism) just the passing of time. It starts when the survival probability curve has reached its maximum (i.e. lowest age-specific mortality rate) and ends with death. However, ageing is fundamentally an event-dependent phenomenon, rather than a time-dependent process (Arking, 2006). Physiological biomarkers are a much more useful index of ageing than is the simple passage of time (Arking, 2006).

Ageing as a Disease. Is ageing a disease? Disease is very much a social construction that takes into account statistical data and available therapy. Regarding statistics, ageing is not a disease because it affects all individuals of a species. Statistically speaking, it is a common feature of most living beings. Regarding available therapy and symptoms, the rate of ageing can be modified but the process is not yet treatable, as all humans eventually die and if they are lucky they die of old age, i.e. the result of ageing.

Developmental Process. Usually each human follows the same path of growth, development, maturity and senescence (Arking, 2006). The changes from maturity through senescence constitute the ageing process (Rothstein, 1982). All the parts of the life cycle are continuous with one another in process and mechanism (Arking, 2006). Ageing is a naturally developing biological process which limits the adaptive possibilities of an organism, increases the likelihood of death, reduces the lifespan and promotes age pathologies (Frolkis, 1982).

Overall, ageing is a process, that involves changes of the structure of an organism. It is multi-factorial and multi-dimensional at its root, occurs gradually, but is degenerative in its consequences as it leads to loss of functionality and ends with death. Ageing could be defined as every irreversible change of living substances as a function of time (Smith, 2002), but are all those processes really irreversible? This seems not to be the case. Most definitions of ageing acknowledge that it is related to changes in the organism leading to a loss in adaptability. This loss in adaptability leads to a higher chance of death. Ageing is a time-independent series of cumulative, progressive, intrinsic, and deleterious function and structural changes that usually begin to manifest themselves at reproductive maturity and eventually culminate in death (Arking, 2006).

1.1.2 Measuring Ageing

It is important to measure ageing, the rate of ageing and the process of ageing. Otherwise it would not be possible to know whether an organism ages faster or slower than others. Nor would it be possible to know whether an anti-ageing intervention is actually working. In practice two different measurement standards are used dependent on whether ageing is measured in a population or in an individual (Arking, 2006).

Ageing in population is measured by using the observed age-specific mortality rates to calculate the number of surviving organisms that will likely die in the next time period. On a demographic level ageing is associated with increased mortality with increasing age as well as increased susceptibility to age-related disease. If the death number increases, then the population is composed of ageing individuals, i.e. ageing population. The rate of ageing can be calculated based on how long it takes for the mortality rate to double (Arking, 2006).

In individuals, ageing is measured by documenting changes in physiological traits, or biomarkers, known to be important to normal functioning and capable of predicting remaining lifespan. The search for such biomarkers of ageing is an important quest of biogerontology. Biomarkers of ageing are usually surrogates of mortality statistics. None have been shown to be predictive of future survival that are better than the age of the individual. A combination of multiple biomarkers (like derived from genomics or other omics) utilized by mathematical models might have the potential to overcome this, e.g. epigenetic markers are promising candidates for being able to reliably predict future mortality (Christiansen, et al., 2016). In the case the observed changes are altered in the direction of loss of function, then the individual is ageing at some calculable rate of loss of function (Arking, 2006).

1.1.3 Plasticity of Ageing

Although ageing is quite widespread across species - it is not an universal phenomenon. Ageing is actually an incredibly plastic process which can be sped up (e.g. by mutation), slowed down (e.g. by dietary restriction), totally stopped (e.g. by genetic knockout), or even reversed (e.g. by overexpression of genes) (Berger, 2014; de Magalhaes, 1997). The exact mechanism of ageing is an enigma. Understanding why certain species exhibit ageing and others do not (negligible senescence and biological immortal species) would revolutionize medicine.

Although defining what exactly is ageing is difficult, yet it is possible to measure it at least reliable on the population level. From this it is clear that the ageing process is quite plastic, which means it varies from species to species and from individual to individual within a species. In fact, there are quite large intraspecies and even bigger interspecies differences in the lifespan of an organism and the rate of ageing. The rate of ageing varies substantially across different species. This is mainly because of genetic differences. Some species live just about a day (e.g. mayfly), while others live centuries (e.g. sharks, whale). Yet others do not appear to have age-related changes, i.e. exhibit negligible senescence (rockfish, lobster, turtle, etc.), and there are even species which have been declared to be immortal (sea squirt, jellyfish, hydra, flatworm, etc.). The naked mole rat might be the first mammal that has been declared to exhibit negligible senescence. Although it lives 9 times longer than similarly sized mice and only shows slight age-related changes with no decline in fertility nor was it observed to develop any spontaneous neoplasm (Buffenstein, 2008), the naked mole rat dies still of old age.

Ageing within a population is not uniform either. There is a genetic heterogeneity as well as environmental influences that lead to a profile of different lifespans.

The ageing process within a single organism can be altered. In particular the genetic makeup and epigenetic modifications play important roles, i.e. genes and their activities affect the ageing process. A variety of alterations in specific genes can extend lifespan quite substantially in model organisms and under laboratory conditions. Those increases are dramatic in simple budding yeast and nematodes, less so in fruit flies and less again in rodents.

Manipulating just single genes can accelerate ageing or slow down ageing hence greatly extend lifespan like up to 10-fold (Shmookler Reis, et al., 2009). It has been demonstrated that it is possible to reverse ageing, even by simple ectopic overexpression of just single transcription factor out of two (*IME1* and *NDT80*) in a model organism such as *Saccharomyces cerevisiae* (Unal, et al., 2011). In humans single gene mutations can lead to accelerated ageing (progeroid syndromes). On the other hand, many genetic differences were found to be associated with longevity in centenarians and some in supercentenarians (Fortney, et al., 2015).

Ageing genes appear to be extremely conserved across species. For example, the sirtuin family of genes has a significant effect on the lifespan in many organisms. Their effect seems to be strong in yeast (Kaeberlein, et al., 1999) and nematodes (Tissenbaum & Guarente, 2001), and weak in fruit flies (Rogina & Helfand, 2004) and mice (Price, et al., 2012). Further a substantial number of non-genetic interventions including single compounds were found to affect ageing and extend lifespan (Vaiserman, et al., 2017).

Ageing is a widespread and almost ubiquitous phenomenon that affects virtually all biological species. For species that age, ageing appears to affect most aspects of an organism in a negative manner and increases its probability of health problems and death with increasing age. Though people live longer today through medical and safety improvements, the ageing process itself still leads to age-related disabilities and causes increasing physical, psychological, and economic burdens. While ageing has been an inevitable phenomenon for centuries, it now becomes more and more of a problem that can be understood (by science) and solved (via technology). Not only could ageing be solved as a theoretical problem, but it could also be treated as a medical condition.

However, the precise mechanisms of ageing remain largely unknown. Despite advancements in understanding the mechanisms of ageing (from a "*backwater*" of biology, full of anecdotes, to a serious discipline) and the possibility to interfere with the ageing process by changing just a single gene, applying dietary interventions (even with a single type of molecule) (de Magalhaes, et al., 2012) or simply repairing the damage (via regenerative medicine), the actual evolutionary theory ("why") and molecular/physiological mechanism ("how") of ageing remains unknown. To understand the biology of ageing requires posing testable and reasonable questions like (Arking, 2006):

- Why do even two closely related species have such very different lifespan, despite their huge physiological similarity?
- What causes the deteriorative changes during the lifespan of each of these species?
- Are the causes the same between various species or are they different?
- Can deteriorative changes be postponed or even reversed?
- Is it feasible to reliable predict individual lifespan?
- Is it possible to prolong the lifespan of humans by utilizing insights from other species?

Some anti-ageing mechanisms are species-specific (private mechanisms), while others appear to be highly conserved across phylogenetic lines (public mechanisms). In the latter case, insights obtained from investigations on one species of laboratory invertebrate organism can be translated to rodents or to primates and perhaps humans. Although all ageing mechanisms are of particular interests, conserved mechanisms have the greatest significance because they may imply possible interventions into human ageing.

Biology does not deal only with mere facts, but rather with developing an understanding of underlying mechanisms. There are three types of evidence (Arking, 2006): 1) Correlative, 2) Loss-of-function, and 3) Gain-of-function.

Loss-of-function and gain-of-function are quite common approaches in genetics, while correlative evidence is dominating genomics. Correlative evidence is derived from observed temporal or spatial correlations between two or more events. Loss-of-function evidence is based on deactivation of an entity, e.g. the inactivation of a gene by knock-out or knock-down. Gain-of-function is the strongest type of evidence in which some technique is used to specifically increase the activity of an entity. All three types of evidence are used to infer causality among events, while it is challenging for correlative evidence, causation is more likely to be inferred from loss-of-function and especially gain-of-function data. However uncertainty of inference remains.

1.1.4 Age-Related Diseases

While humans died in the past mostly in the perinatal period (i.e. death in the first five years of life) or due some infectious disease, today people suffer from age-related diseases and more than 100,000 individuals die daily due to the consequences of the ageing process, either directly (as a primary cause via age-related diseases) or indirectly (as contributory cause due to reduced functionality) (de Grey, 2007; YourTribute, 2013).

Ageing is the underlying cause of almost all major human diseases, such as atherosclerosis, cancer, cardiovascular defects, cataract, type 2 diabetes, dementia, macular degeneration, neurodegeneration, osteoporosis and excessive muscle loss leading to sarcopenia (Rattan, 2007; Holliday, 2017). Ageing is by far the leading cause of death (though it acts mostly indirectly and in a gradual manner). Of the 150,000 people dying every day, two thirds die of age-related disease. The proportion is even higher in industrialized nations where it is reaching 90% (de Grey, 2007).

Ageing is directly associated with numerous age-related diseases (e.g. neurodegeneration, metabolic and cardiovascular diseases, cancer, etc.) and the single risk factor common to all of the leading causes of death is age (Hayflick, 2007; Fontana, et al., 2014). Therefore the research in animals and humans should be refocused to find ways to prevent and treat ageing (Fontana, et al., 2014). Slowing down the ageing process by even the slightest degree could dramatically increase the general health much more than the elimination of any single disease. Hence understanding ageing as the root cause of many diseases would be far more efficient than tackling age-related diseases one-by-one (Hayflick, 2007; Fontana, et al., 2014). If ageing could be slowed down even slightly the improved health of humans and the economic benefit would be significant.

The research on numerous diseases has demonstrated that an underlying risk factor for many illnesses is age. In fact, the single risk factor common to many diseases is age. The susceptibility to the effects or severity of a disease is significantly modified by ageing. Further, chronological age is the most important risk factor for the development of diverse diseases, including degenerative diseases and cancer (Brett & Rando, 2014). Ageing causes disease and loss of function that in turn causes death. The difficulty with this is that one is more or less forced into defining the boundary where the disease or loss-of-function begins, which is almost impossible because it always begins very subtly. It is therefore useful to define ageing as the *über-disease* encompassing all late-life dysfunctions, such that disease and functional deficits of old age are simply facets of the later stages of ageing - they are part of ageing rather than being distinct from it. Thereby ageing does directly cause death, and one can subdivide the specific sequences of events that lead to death in a given individual.

Whether ageing should be acknowledged as a disease is debatable (Gems, 2015; Bulterijs, et al., 2015). Arguments for this acknowledgement are that 1) ageing actually fits very well most definitions of disease, and 2) this acknowledgement could have an impact on making it easier to develop anti-ageing drugs.

On the contrary, one of the arguments against defining it as a disease is that it is almost universal, i.e. it seems to occur in every member of a species. However this universality is questionable as there exist species that do not appear to show population ageing. Another counterargument is that it is normal as it occurs in every human, while disease is more often associated with abnormality. However, an infectious disease, like one caused by a virus, could theoretically affect most or even all individuals of a population or perhaps a species. Although having this disease is the norm, it is still a disease. Quite often ageing is semantically understood as a neutral term (especially in English) that just means the passage of time and includes development. However, it still has a negative sentiment and often refers to the later phase of life that is associated with deterioration which some people refer to as senescence. One could also argue that

there is no point in calling something a disease as it can not yet be treated. But this is not true as there are already interventions that counteract ageing and on the other hand there are also diseases that have not yet any cure and can not really be treated. Others argue often that ageing-related changes are not always negative and that it also has positive aspects. Overall this does not hinder its classification as a disease as diseases are also associated with certain changes that have no negative influences (or are not known to be negative). Sometimes the complexity and heterogeneity of the phenotypes or symptoms of ageing are used as a counterargument to classifying ageing as disease, because diseases often have very well-defined symptoms. This argument ignores the heterogeneity of humans themselves and the fact that a disease also affects individuals differently depending on their genetic disposition and lifestyle as well as environment. Yet, another argument states that ageing requires research on things that maintain health and everything that increases health counteracts ageing. However most likely even when living as healthily as possible by any current recommendations individuals will still age and die.

If ageing was to be declared as a disease, it would have little effect on the scientific quest of seeking the underlying molecular mechanisms that cause and control the rate of ageing, it may however make it easier to develop drugs that counteract ageing as an indication rather than some specific age-related diseases.

Overall there are no contradictions in defining ageing as a disease, although ageing IS NOT a single disease as it manifests itself in many different sub-diseases. It is somehow an *Überdisease*. It is the root cause and major risk factor of many diseases. One can conversely view each age-related disease as only one symptom of the overarching master disease commonly referred to as "ageing". Even if one symptom could be cured the next age-related disease would strike soon as long as its main underlying causative mechanism, i.e. ageing, is not addressed.

1.1.5 Age-Related Changes

There are myriads of changes happening during ageing but only few might be the drivers of the process while others are merely symptoms. Ageing is not simply the sum of the aggregate pathologies and of disease-induced changes, and conversely, not all the changes in structure and function that are correlated with age may be appropriately considered as fundamental age-related changes (causes) per se. A fundamental age-related change must meet the following four conditions (Strehler, 1982):

1. Deleterious, reduce function
2. Progressive, take place gradually
3. Intrinsic, not the result of a modifiable environmental agent
4. Universal, all members of a species should exhibit such gradual deficit with advancing age

However the concept of universality seems to be invalid as there is so much individual variation in ageing due in part to our genetic heterogeneity and in part to chance alone (Finch & Kirkwood, 2000) that it is not possible for all members of a species to exhibit ageing in an identical manner. The concept of intrinsic change, while being valid, is being narrowed in light of the fact that various lifestyle practices modulate events that were assumed to be completely intrinsic. Nonetheless the concept of deleterious progressive and intrinsic changes is useful, such as in the investigation of stem cell ageing or cellular senescence.

1.1.6 Mechanisms of Ageing

There are many possible mechanisms involved in ageing. Over the last decades, theories about ageing have emerged and faded. The true nature of the ageing process however, is still uncertain. What is exactly happening on a biochemical, genetic, and physiological level still remains to be understood. What we see in an ageing person is a great deal of change in appearance, a decline in overall fitness and an increased chance of getting age-related diseases. These are, however, only the visible sides of the changes that start at the molecular level, then the cellular level, then progress to the tissue and organ level until in the end the whole physiology is affected. What are these processes that result in ageing? Although ageing is undoubtedly complex, it may be regulated by common mechanisms that are simpler than the effects they produce (Arking, 2006).

Defining ageing clearly is challenging. Our understanding of how and why we age will originate from the study of the processes that happen in the cell and observable biological markers of ageing.

1.3 Cellular Senescence

Cellular senescence is an irreversible growth arrest that may be an evolved antitumour mechanism. Senescence is a natural mechanism restricting propagation of cells, which passed through genotoxic or oncogene stresses, by epigenetic reprogramming. It is a kind of program or a state, basically subroutine, which the cell adapts after damage, stress or some kind of damaging signal. Senescence is a general purpose mechanism that is used for repair systems. Cellular senescence prevents the proliferation of preneoplastic cells and has beneficial roles in tissue remodelling during embryogenesis and wound healing (Baker, et al., 2016). It is a primitive and very ancient process that even occurs in single cellular organism.

Cellular senescence is induced by short telomeres (replicative senescence), but it can also be induced by various stressors (stress-induced premature senescence). There are more than 50 oncogenic or mitogenic alterations that are able to induce senescence (Gorgoulis & Halazonetis, 2010).

There are certain phenotypes associated with the state of cellular senescence such as obvious morphological changes. Senescence cells enlarge and become resistant to apoptosis. Cellular senescence is frequently characterized by the expression of the p16 protein. There is also the formation of senescence-associated heterochromatin foci.

Cellular senescence exhibits a distinctive secretory phenotype (Baker, et al., 2016). This associated secretion profile (Senescence-Associated Secretory Phenotype; SASP) of senescence cells depends on the type of insult. Senescence markers show already up during development. However senescence cells accumulate in various tissues/organs over time (Baker, et al., 2016). It is a growth arrest that for some reason occurs because either the cell is damaged or due to some regenerative response. The type of profile of secretion depends on the context of damage/triggering signalling.

It is commonly believed that cellular senescence underlies organismal senescence in mammals.

1.4 Biomarkers of Ageing

Ageing is a dynamic process that does not only depend on the species, the genotype and phenotype, but is also dependent on environmental influences. The ageing/age of an individual can not yet be directly determined.

Biomarkers of ageing are biomarkers that better predict functional capacity at a later age than chronological age (Baker & Sprott, 1988). Biomarkers of ageing would give the true biological age, which may be different from the chronological age. Validated biomarkers of ageing would allow for testing interventions to extend lifespan, because changes in the biomarkers would be observable throughout lifespan of the organism (Baker & Sprott, 1988). Ideally, biomarkers of ageing should assay the biological process of ageing and not predisposition to disease, should cause a minimal amount of trauma to assay in the organism, and should be reproducibly measurable during a short interval compared to the lifespan of the organism (Baker & Sprott, 1988).

Although greying of hair increases with age (Van Neste & Tobin, 2004), hair greying cannot be called a biomarker of ageing. Similarly, skin wrinkles and other common changes seen with ageing are not better indicators of future functionality than chronological age. Biogerontologists have continued efforts to find and validate biomarkers of ageing, but success this far has been limited. Although maximum lifespan would be a means of validating biomarkers of ageing, it would not be a practical means for relatively long-lived species such as humans.

Levels of CD4 and CD8 memory T cells and naive T cells have been used to give good predictions of the expected lifespan of middle-aged mice (Harrison, 2011; Miller, 2001). Biological clocks, for example the epigenetic clock, are promising biomarkers of ageing (Horvath, 2013). Ideally changing this biomarker itself is known to increase the lifespan. Though this is not necessary. The methods should be non-invasive or as less invasive as possible. If erythrocytes can be obtained, one could for instance measure glutathione disulfide/glutathione (GSSG/GSH) ratios (i.e. oxidation state of glutathione). Also circadian parameters might be interesting, as the circadian clock is disturbed as one gets older and interventions that extend the lifespan, such as dietary restriction, make this clock tick much more robust. It is of particular interest to establish methods that can be automated so that they are easy to be taken continuously or periodically and can be scaled to many individuals. A comprehensive inventory of possible biomarkers of ageing need to be established to better assess the impact of established or potential novel manipulations of ageing.

1.5 Dietary Restriction

1.5.1 Background

The most powerful non-genetic intervention to retard the biological ageing process is commonly known as dietary restriction (DR) which includes calorie/caloric restriction (CR) and other form of restricted diets. A reduction in dietary intake without malnutrition robustly extends the lifespan of many species from yeast to rodents. It is the only regimen that extends the lifespan and healthspan in as wide a spectrum of organisms as yeast, nematodes, mice, rats, dogs, and possibly non-human primates (Colman, et al., 2009; Mattison, et al., 2012; Weindruch, et al., 1986).

Dietary restriction can slow down ageing and extend youthful healthy lifespan in evolutionarily very distantly related species from unicellular lifeforms (yeast), to invertebrates (flies, spiders, rotifers, nematodes), rodents (mice, rats), dogs, and possibly rabbits and monkeys. Restriction of energy intake extends the lifespan of mice and rats relative to nonobese *ad libitum* controls up to and including a 50% reduction, which increases lifespan by approximately the same proportion. As a side effect, many age-related diseases (neurodegeneration, metabolic disease, cardiovascular disease and cancer) and other changes are effectively postponed, progression decelerated, or even prevented totally. Although DR does not stop the ageing process, it definitely delays and/or retards the ageing process in many experimental animals (de Magalhaes, et al., 2012).

There are several DR regimens for which some evidence of age-retarding effects exists.

1.5.2 Regimens

Lifespan extending DR regimens can be quantitative (caloric intake), qualitative (particular nutrients) or temporal (fasting) in their very nature:

- Calorie Restriction
- Protein Restriction
- Amino Acid Restriction
    - Tryptophan Restriction
    - Leucine Restriction
    - Methionine Restriction
- Water Restriction
- Intermittent Fasting

Dietary restriction (DR) is a non-genetic intervention that slows down ageing across taxa. DR has clear positive effects on nematodes, flies and inbred mouse strains as well as rats. However results in

wild-caught mice, primates and human populations indicate that the genetic background, the age of onset of DR, and the composition of the diet affect the longevity-promoting effect in mammals (Swindell, 2012).

DR of total food intake, calorie or caloric restriction, robustly extends lifespan and hence implies that the energy intake is the crucial factor, but intriguingly restricting only particular nutrients, notably some specific amino acids, was also effective in slowing down ageing to an extent comparable to CR, without a reduction in total caloric intake (Grandison, et al., 2009; Wu, et al., 2013; Lee, et al., 2014; Min & Tatar, 2006; Emran, et al., 2014; Troen, et al., 2007). This raises the possibility that CR extends lifespan actually by lowering the level of specific nutrients (e.g. amino acids). Alternatively it is also possible that energy intake is not the only way to slow ageing and extend lifespan by dietary modulation. It is therefore logical to assume that diverse DR regimens either control the same effectors modulating ageing or different regimens may modulate different ageing systems. Nevertheless, by comparing different DR regimens which are effective in retarding ageing it will be possible to dissect the common affected processes (drivers) from the less important ones (passengers).

A diet containing a third less calories than usual extends the lifetime of mice and other mammals by up to 40% and drops their body temperature by half a degree or more (Pearson, 2006). A calorie restricted diet lowers IGF1 levels, promotes apoptosis over cell proliferation and slows down tumour progression. Restoring normal *ad libitum* IGF1 levels in mice under DR abrogates the protective effect of DR in particularly on neoplastic progression (Dunn, et al., 1997).

Neither a reduction in fat nor in mineral content altered survival of rats under constant energy intake (Iwasaki, et al., 1988b; Kubo, et al., 1987; Shimokawa, et al., 1996). But, by reducing the total amount or just the amount of a particular nutrient such as carbohydrate (in yeast, worm, fly and rats), proteins (in rats and mice) or amino acids (in yeast, fruit fly, mouse and rats) lifespan was extended (Place & Cruickshank, 2010).

1.5.3 Caloric Restriction

60% CR in mice increased lifespan by up to 65% (Weindruch, et al., 1986). CR increased lifespan in species as diverse as fruit flies (Min, et al., 2007), guppies (Comfort, 1963) and dogs (Lawler, et al., 2008). CR exerts beneficial effects including delayed immune senescence, retardation of cancer development, alteration in gene expression, improved antioxidant protection and enhanced DNA repair (Orentreich, et al., 1993). CR increases insulin sensitivity (Wang, et al., 2009) and heart function, and decreases inflammation and muscle wasting of ageing. It also prevents age-related diseases such as cardiovascular diseases (Willcox & Willcox, 2014; Lopez-Lluch & Navas, 2016), metabolic disease (Lopez-Lluch & Navas, 2016), neurodegeneration (Willcox & Willcox, 2014) and cancer (Weindruch, 1992; Willcox & Willcox, 2014). In essence, it is effective against many kind of diseases especially age-related diseases. It may be interesting to identify any age-related physiological change or disease that is not affected by DR. This might give insight into its mechanism of action since the obvious question would be, why are these changes/disease not affected by DR?

CR mice have a higher metabolic rate. Therefore, the anti-ageing action of CR is not due to a reduction in metabolic intensity or to a decreased intake of energy per unit of lean body mass, but rather involves a total organism response involving the nervous and/or endocrine system (Masoro, et al., 1991). CR initiated after the age of 6 months (almost completely matured) was as effective as at 6 weeks of age in extending lifespan in rats (Yu, et al., 1985), indicating that CR slows the growth and development program in adulthood (Masoro, et al., 1991).

CR alone is sufficient to extend lifespan. Parasitoid wasps have a simple diet consistent of carbohydrate only. Only dietary dilution showed an effect with highest longevity at 80% sucrose (w/v), while there was no effect on fecundity (Ellers, et al., 2011).

1.5.4 Protein Restriction

Protein restriction extends lifespan in yeast and flies, but it was originally not clear that it does so in rodents aside from as a side effect of CR (Iwasaki, et al., 1988a). 40-85% protein restriction increases maximum lifespan by up to 20% in 16 out of 18 studies in rodents (Trepanowski, et al., 2011; Pamplona & Barja, 2006; Leto, et al., 1976; Goodrick, 1978; Stoltzner, 1977; Fernandes, et al., 1976; Barrows & Kokkonen, 1975; Yu, et al., 1985; Horakova, et al., 1988; Davis, et al., 1983; Ross & Bras, 1975; Miller & Payne, 1968; Ross, 1961).

The type of protein fed to rats has an impact on lifespan. For instance substituting soy protein for casein protein in rat diet increases the mean, median and maximum lifespan while caloric intake was the same (Gilani, et al., 2009).

1.5.5 Methionine Restriction

Methionine restriction (MR) extends lifespan in flies, mice and rats and a couple of other species, but is not as widely documented as CR.

Methionine restriction extended lifespan in flies (Grandison, et al., 2009; Troen, et al., 2007; Lee, et al., 2014), mice (Miller, et al., 2005; Sun, et al., 2009) and rats (Orentreich, et al., 1993; Richie, et al., 1994; Zimmerman, et al., 2003). Lifespan in *Drosophila* can be modified by varying the glucose and methionine concentration in a chemically defined diet (Troen, et al., 2007).

80% MR (0.86 to 0.17%) restriction extended lifespan by 30% in rats. MR abolished growth, although food intake was actually greater on a body weight basis. Increasing the energy intake of MR rats failed to increase their rate of growth, whereas restricting control to food intake of MR rats did not materially reduce growth. Thus, food restriction (i.e. decreased calories) was not a factor in lifespan extension (Orentreich, et al., 1993). Methionine restriction resulted in 42% increase in mean and 44% increase in maximum lifespan in rats (Richie, et al., 1994).

Methionine restriction lowered IGF1, thyroxine (T4), insulin (25%) and fasting glucose (50%) (Miller, et al., 2005). MIF (migration inhibition factor) mRNA are increased in young adult mice of long-lived Snell dwarf and Ghr KO mice as well as under CR (Miller, et al., 2002). Methionine restriction also increased mRNA and the protein concentration of MIF (Miller, et al., 2005).

Food restriction limited to the first 20 days of life extended median and maximal lifespan. Methionine restriction initiated at 12 months in mice increases mean, median (7% increase), and maximum lifespan. CR mice had increase in phosphorylation of Erk, Jnk2, and p38, as well as a decrease in phosphorylation of Akt, mTOR, and 4Ebp1. Methionine restricted mice did not exhibit a decrease of phosphorylation at mTOR and 4Ebp1 (Sun, et al., 2009).

MR downregulates IGF1 and upregulates IGFBP1 (Takenaka, et al., 2000). Methionine supports protein synthesis and has the capacity to maintain IGF production (shared by all essential amino acids), function as a methyl donor (via S-adenosyl methionine; SAM) and as precursor for taurine, polyamines, glutathione and sulphate. MR protocols have not included cysteine or most other non-essential amino acids (McCarty, et al., 2009).

Lowering the content of sulfhydryl-containing amino acids by removing cysteine and restricting the concentration of methionine extend all parameters of survival and maintain blood levels of GSH without a reduction of energy intake (Zimmerman, et al., 2003). Methionine restriction exerts its beneficial effect, e.g. increased adiponectin and triiodothyronine without energy restriction (Malloy, et al., 2006). Although MR may not be associated with energy restriction, if protein synthesis is affected by methionine restriction, the digestion, absorption and metabolic use of dietary energy may be affected. Cysteine supplementation can reverse positive effects mediated by methionine restriction (Elshorbagy, et al., 2011).

80% MR in rodents (without CR), like DR, increases maximum longevity and strongly decreases mitochondrial ROS generation and oxidative stress as well as lowers the degree of membrane fatty acid unsaturation in rat liver. Similar 40% MR in rats decreases mitochondrial ROS production and percent free radical leak at complex I during forward (but not during reverse) electron flow in brain and kidney

mitochondria. MR induces changes to mitochondria in rat brain and kidney similar to caloric and protein restriction (Caro, et al., 2009).

1.5.6 Tryptophan Restriction

30-40% tryptophan restriction starting from weaning can delay ageing in Long-Evans females rats. The mortality was greater in the juvenile period, but substantially less than normal if fed at late ages (Ooka, et al., 1988). Mice on a tryptophan restricted diet exhibit greater survival and reduced growth (De Marte & Enesco, 1986).

The effect of tryptophan restriction is somewhat unclear in rodents, as it causes very high early mortality, and is again not as widely documented as CR. Tryptophan restriction is confounded by involuntary CR (as measured by reduced food intake and/or body weight) in all of the cases.

1.5.7 Water Restriction

Water restriction implemented similar to the protocol for the original caloric restriction experiment (McCay, 1933) extends lifespan of rats (Sprague-Dawley females from Taconic labs) even more than caloric restriction (Clifton, 2010). Usually it assumed that it is detrimental not to have *ad libitum* water and it would be very healthy to drink a lot of water. From an evolutionary point of view it makes sense that drought (when water resources are limited) should delay reproduction and ageing.

The lifespan extending effect could be due to "voluntary" caloric restriction, as the lack of water could just be inducing them to eat less. But caloric restriction might be causing animals also to drink less; CR studies normally do not control for water intake. Replication of lifespan experiments under water restriction is required.

1.5.8 Intermittent Fasting

Prolonged fasting started at middle-age extends longevity, lowers visceral fat, reduces cancer incidence and skin lesions, rejuvenates the immune system, and retards bone mineral density loss (Brandhorst, et al., 2015). In rodents intermittent fasting only works as a side-effect of CR (the degree of lifespan extension is exactly what one would expect from the degree of unintentional reduction in energy intake) (Anson, et al., 2003).

1.5.9 Invertebrates

In yeast caloric restriction is usually applied by reducing the sugar content from 2% (*ad libitum*) to 0.5% (moderate CR) or even to 0.05% (severe CR). Though there is also extreme CR which is basically starvation in water without any sugar added.

Genetic manipulations in nutrient-signalling pathways mimic the effects of dietary restriction. It seems that caloric restriction requires mitochondrial respiration to increase longevity (chronological ageing), but not always (for replicative ageing) (Kaeberlein, et al., 2007; Dilova, et al., 2007; Chen & Guarente, 2007; Piper, 2006; Longo, 2009; Longo, et al., 2012).

In the nematode, dietary restriction can be accomplished via a variety of protocols like reducing bacteria on liquid or solid media in plates. In fact, multiple methods of DR are used in *C. elegans*, including mutation that reduce pharyngeal pumping (food intake impaired *eat-2* mutants), removal of the bacteria food source, dilution of live or dead bacteria, and axenic culture (Mair & Dillin, 2008).

Several dietary restriction regimens can extend the lifespan of fruit flies. Different laboratories use different diets and techniques to implement DR. However DR seems to fail in species like medflies and houseflies (Szafranski & Mekhail, 2014).

1.5.10 Vertebrates

1.5.10.1 Rodents

Reducing the food consumption 25-60% without malnutrition extends the lifespan of rodents up to 50% (Weindruch, et al., 1986) and delays the onset of age-related maladies (Colman, et al., 2009; Koubova & Guarente, 2003). In mice and rats, DR can extend longevity by up to 50%, delay physiological ageing and postpone or diminish the morbidity of most age-related diseases (Masoro, 2005). DR elicits major metabolic reprogramming towards efficient fuel utilization and the reduction in oxidative damage to macromolecules (Anderson & Weindruch, 2012; Sohal & Weindruch, 1996). DR triggers global reprogramming of mitochondrial protein acetylome (Hebert, et al., 2013). DR has to be applied continuously to produce the effect on maximum lifespan. If rats are refed at least up to 12 months of age, all the effects of DR on extending survival is lost. Therefore DR is acting as some type of metabolic brake.

Mice placed under CR live longer and exhibit resistance to age-associated diseases (Omodei & Fontana, 2011; Trepanowski, et al., 2011; Fontana, et al., 2010). These mice also consume most of their food within a few hours, however their peripheral clocks are well synchronized with the suprachiasmatic nucleus (which determines time of day in mammals) (Froy & Miskin, 2010). The synchrony observed in mice under CR could be the source of lifespan extension (Froy & Miskin, 2010). Longevity-conferring diets cause major metabolic changes in normal mice, but not in mice whose growth hormone receptor was knocked out (Westbrook, et al., 2014). Some inbred mouse strains do not live longer on DR. This has been found to be the case for DBA/2 male mice (Hempenstall, et al., 2010) and several strains among the ILSXISS recombinant inbred panel. DR does shorten the lifespan in even more ILSXISS strains than it extends (Liao, et al., 2010). Dietary restriction does not appear to extend the lifespan of wild mice (Harper, et al., 2006). All rodent studies might be biased to the effects of laboratory breeding (Swindell, 2012). However the problem with these studies using the ILSXISS panel of mice is that too few animals (about 8-12) were used to determine lifetime survival in each group. About 50, preferably over a 100 are needed for robust survival statistics in lifespan studies.

Reducing the amount of calories fed to rats nearly increases their mean and maximum lifespan by up to 50%. In rats, DR increases median lifespan by 14-45% in half of all experiments (Swindell, 2012). Severe DR extends lifespan of rats by nearly 50% (Abalan, et al., 2010).

1.5.10.2 Primates

Whether DR also prolongs lifespan in primates is questionable. Studies in monkeys and humans indicate health benefits associated with a restricted diet and maybe also an extension of the lifespan.

1.5.10.2.1 Monkeys

Initial studies in rhesus monkeys indicate a longer lifespan, due to an overall reduced mortality (Wisconsin study) (Colman, et al., 2009). However this was only true if censorship due to non-age-related diseases was applied to the survival data. Another study conducted by the "National Institute of Aging" (NIA) did not reveal any average lifespan benefit from a 30% DR in rhesus monkeys. DR improves some test results, but only in monkeys put on the diet when they were old. Still an effect on maximum lifespan remains unknown (Kolata, 2012; Mattison, et al., 2012).

The Wisconsin monkeys received a much higher sucrose content in their food than the NIA animals. Wisconsin control animals could eat as much as they like, while the NIA control were given a set amount of food.

1.5.10.2.2 Humans

When done correctly, DR appears to confer health benefits for humans, such as lowering the risk of heart disease. Moderate CR in humans ameliorates multiple metabolic and hormonal factors that are implicated in the pathogenesis of age-related disease such as type 2 diabetes, cardiovascular diseases, and cancer (Most, et al., 2016). People on DR have hearts that function more like those found in people two decades younger (Stein, et al., 2012).

There are three types of evidence that DR works in humans:

1. Okinawa Diet
2. Biosphere 2
3. Caloric Restriction Society

1.5.10.2.2.1 Okinawa Diet

First of all, inhabitants of Okinawa (a Japanese island) practice a DR-like lifestyle as part of their culture, and Okinawans enjoy simple lives (Morgan, 2003). Okinawans are the longest-lived (Willcox, et al., 2007a) and most disease-free population (Bernstein, et al., 2004) on the globe. Okinawa has a high centenarian prevalence (Willcox, et al., 2008a). In fact, Okinawa has the highest prevalence of exceptionally long-lived individuals in the world (Willcox, et al., 2006a). Okinawan centenarians exhibit a high functional status throughout their 90s (Willcox, et al., 2007b).

Okinawa centenarians have low plasma lipid peroxide implying protection against oxidative stress. Vitamin E tocopherols is also lower in Okinawa centenarians, but intracellular beta-tocopherol is higher. tocopherol/cholesterol and tocopherol/lipid peroxide (LPO) ratios were not different between age groups, although there was a correlation between α-tocopherol and LPO in septuagenarians but not in centenarians (Suzuki, et al., 2010). Epidemiological analysis on Okinawans found a low-caloric intake and negative energy balance at younger ages, little weight gain with age, lifelong low body mass index (BMI), relatively high plasma dehydroepiandrosterone (DHEA) levels at older ages, low risk for mortality from age-related diseases, and survival patterns consistent with extended mean and maximum lifespan (Willcox, et al., 2007a).

Epidemiological evidence indicates that CR might have contributed to an extension of average and maximum lifespan and lowered risk for age-associated chronic diseases (Willcox, et al., 2006b). Okinawa supercentenarians display an exceptionally healthy phenotype where clinically apparent major chronic diseases and disabilities were markedly delayed, with little clinical history of cardiovascular disease and no history of cancer or diabetes (Willcox, et al., 2008b).

Whether the remarkable longevity of Okinawans is mainly due to their diet or because of their genetics is still an open question. This could be resolved by studying any Okinawans who migrated to another country and adopted the diet of the new country. Such studies have been used to show the effect of diet on morbidity rates in Japanese subjects who emigrated to the USA.

1.5.10.2.2.2 Biosphere 2

Secondly, the Biosphere 2 crew, who lived in isolation for two years, had a low-caloric diet and experienced many physiological, haematological, hormonal and biochemical (e.g. Insulin, T3 glucose and cholesterol decreased) changes, which resemble those of rodents and monkeys maintained on DR and remained in excellent health and high physical and mental activity. Additional variations in several substances, not hitherto studied in DR animals, like androstenedione, thyroid binding globulin, renin, and transferrin were also observed (Walford, et al., 2002).

The problem with this study is that the subject numbers were so low and the experiment had to be terminated when they could not produce sufficient food to support survival.

1.5.10.2.2.3 Caloric Restriction Society

Thirdly, ongoing studies on dietary restricting human volunteers (e.g. Caloric Restriction Society) already showed that it exerts beneficial effects on health and that, in particular, moderate protein restriction evokes similar adaptive responses as in dietary restricted rodents and monkeys (Fontana, et al., 2008).

The *Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy* (CALERIE) research program is a systematic investigation of dietary restriction in nonobese human individuals (Rochon, et al., 2011). It was found that the dietary restricted humans had lower insulin resistance, lower LDL cholesterol levels, lower body temperature and blood insulin levels as well as less oxidative damage to the DNA (Economist, 2006).

CR decreases serum IGF1 (40%), protects against cancer and slows ageing in rodents. While severe CR without malnutrition did not change IGF1 and IGF/IGFBP3 ratio levels in humans, total and free IGF1 were lower in moderately protein restriction (1.67 to 0.95g/kg body weight per day) (Fontana, et al., 2008).

1.5.10.2.3 Caloric Intake

It is not yet know what the optimum of food intake is and which factors are most important to restrict and, as one can easily assume, these vary by species as well as strains and in humans by individuals. For humans, the usual recommended diet for adult males is about 2500 calories/day.

The caloric intake of Okinawans was 1785 kcal/day (Willcox, et al., 2007a), which is 15% and 40% less than the average of mainland Japanese (2068 kcal/day) and US (2980 kcal/day), respectively. Biospherians consumed 30% less (from 2500 to 1784 kcal/day) for the first 6 months and then 2000 kcal/day for the remaining 12 months, which was combined with high level of physical activity of 70-80 hour work/week (Walford, et al., 1992). Caloric Restriction Society members eat about 1800 kcal/day, which is 30% less than a typical Western diet (Holloszy & Fontana, 2007).

1.5.11 Side Effects

In humans a severely restricted diet can lead to low testosterone levels and problems with maintaining bone density in male individuals (Naik, 2012). A moderately restricted diet is assumed to not reduce the quality of life. Rodents under DR are usually more active on the physical and mental levels even into advanced ages. Volunteering people practising DR report that they experience much higher concentration levels.

It is actually exactly the opposite; eating too much reduces the quality of life, making individuals tired during the daytime period and causing problems sleeping well during the night time. However severe or extreme DR may result in serious deleterious effects.

1.5.12 Mechanism

Dietary restriction is associated with a variety of changes like metabolic, transcriptional and epigenetic reprogramming. Those changes can be used as surrogates for biomarkers of longevity. How dietary restriction interferes with ageing is still unknown but several potential mechanistic explanations exist.

Genome Stability. Mice under dietary restriction accumulate longer telomeres (Biocompare, 2013). Dietary restriction seems to attenuate telomere erosion (Vera, et al., 2013). CR promotes genomic stability by increasing DNA repair capacity, specifically base excision repair (BER) and completely reverses age-related decline in BER capacity in many tissues (brain, liver, spleen and testes), which is accompanied by a reversal in age-related loss of β-pol protein, mRNA and activity levels. BER capacity, β-pol protein and mRNA were already upregulated in young (4-6 months) (Cabelof, et al., 2003).

Hormesis. DR extends median and maximal lifespan. It does it actually by slowing down ageing. Starvation is the most extreme form of malnutrition and when prolonged can seriously damage an organism. However, the DR response has some similarities to starvation and it was proposed that DR (i.e. low nutrient state) utilizes a low level of stress which enhances defences and repair systems. These beneficial effects of a low stress stimulus is conceptualised as “hormesis”.

Genetics. The longevity promoting effect of DR seems to be mediated by specific signalling pathways. Defined genes appear to be essential for the lifespan-extending effect. Genes that mediate the effect of DR on longevity are designated as DR-essential genes. Sequences of DR-essential genes do not vary across species, but rather they appear to be conserved. It is probable that DR-essential genes are likely to change their activity under DR (i.e. become up- or downregulated). Upon DR, Insulin/IGF1/GH axis and TOR signalling are downregulated, while AMPK, sirtuins and FOXOs become upregulated.

Epigenetics. Epigenetic mechanisms have been recognized as major contributors to nutrition-related longevity and control of ageing. Dietary restriction affects DNA methylation and histone modifications via activation of DNA methyltransferases and histone remodelling which primarily includes histone acetylation and methylation. This in turn reverses certain ageing gene expression changes and ensures the

maintenance of the chromatin stability, resulting in delay of ageing and age-related diseases (Li, et al., 2011).

Evolution. The most accepted evolutionary explanation of dietary restriction is that in times of famine when nutrients are limited, organisms trigger a specific genetic program to slow down ageing and reproduction in order to survive. A restricted diet might also trigger the higher turnover of molecules, cells and tissues and therefore provoke a light form of rejuvenation.

A variety of dietary regimens have been shown to be effective in interfering with the ageing process in multitude of evolutionary distinct species. How DR interferes with ageing and which kind of regimens work well in human is not yet certain. As DR works in almost all species (but not all strains/individuals) tested so far, it would be really surprising if it did not work at all in humans. It is therefore hoped that research on DR will reveal which factors in the diet are crucial for a healthy lifestyle. By making policy-makers aware of it, they could identify and define healthier diets. Further, understanding the actual mechanism underlying DR's anti-ageing effect could lead to the identification of supplements or even development of pharmacological products mimicking the effect of DR without severely restricting one's diet (i.e. DR mimetics).

Research on dietary restriction might enable the identification of genes and molecules that mimic the effect of dietary restriction without the necessity to restrict the diet. Dietary restriction mimetics are genetic interventions or components (i.e. pharmacological interventions) that mimic the biochemical or functional effects of dietary restriction (Madeo, et al., 2014). The aim of research on dietary restriction is not enabling of living longer, but being in a young and youthful state. Staying youthful and healthy longer is definitely not a wrong aim. Generally, a low-caloric, nutrient-dense, diversified diet low in fat and proteins (preferential mainly plant-derived) and without any extra added sugar is beneficial. However, for interfering with the ageing process with a greater extent powerful anti-ageing drugs are needed.

1.6 Anti-Ageing Drugs

1.6.1 Background

Medicaments could one day slow down and reverse the ageing process in humans. Among the most ignored medical findings are that the biological ageing process is the greatest risk factor for life-threatening diseases such as atherosclerosis, cancer, dementia, and diabetes (Perez-Jimenez, et al., 2005). Logically it follows that it should be interfered with the ageing process, instead of trying to treat one disease after the other. Anti-ageing strategies can therefore be viewed as an extension of medicine.

A review of recent research results indicate the following: Firstly, defined genetic mutations can prolong the lifespan and delay age-related disease (Mari, 2011). These genes could be targeted by therapeutics. A change in the receptors for growth factor hormone can for instance increase the lifespan of mice and inhibit tumourigenesis in humans (Coschigano, et al., 2000; Bonkowski, et al., 2006; Arum, et al., 2009; List, et al., 2011; Coschigano, et al., 2003; Zhou, et al., 1997; Kinney, et al., 2001; Guevara-Aguirre, et al., 2011). Secondly, in humans who are older than 100 - a feature that is partly inheritable - age-related diseases occur in later years (Deluty, et al., 2015). Thirdly, a significant reduction in caloric intake extends the lifespan and delays the incidence of chronic diseases in a range of animal species (Tocchetti, et al., 2010). Fourthly, compounds such as metformin (Martin-Montalvo, et al., 2013), acarbose (Harrison, et al., 2014), and rapamycin (Anisimov, et al., 2011a) among others extend the lifespan and delay the occurrence of cancer and dementia in mice. From these observations it is possible to draw the conclusion that age-related diseases can be treated all at once, by modifying the basic ageing process using a therapeutic approach.

There are several major research programs to experimentally test compounds to determine their effect on ageing. The US National Institute of "Aging's Intervention Testing Program" (ITP) is a multi-institutional study on investigating treatments with the potential to extend lifespan and delay age-related disease and dysfunction by utilising lifespan experiments in mice (Nadon, et al., 2016). Secondly, a more recent

international effort known as the Major Mouse Testing Program (MMTP) has been initiated from crowd funding campaigns (MMPT, 2016).

Drugs that counteract ageing are often referred to as anti-ageing drugs or geroprotectors. Geroprotectors are therapeutics that aim to affect the root cause of ageing and thus treat age-related diseases all together, and therefore prolong the lifespan of organisms including humans (Fedichev, et al., 2011).

Modification of the genome is very risky and extremely difficult to perform with prevision. Compounds that interfere with gene activity and metabolism are a more adequate approach.

1.6.2 Drug Discovery

A number of bioactive agents have been identified to have promising "anti-ageing" (geroprotective) effects. Usually a substance is considered to have ageing modulating activity if its efficiency can be confirmed *in vitro* or *in vivo*.

A compound, or an intervention more generally, can be found to slow down the progression of a certain age-related change or reverse the change by reducing the quantity of this change / these changes. However, genuine anti-ageing drugs need to fulfil the criteria of being capable of extending the lifespan of an organism and especially prolonging the maximum lifespan.

Anti-ageing drugs can be identified in a variety of ways. One way is via molecular interactions with the protein products of ageing-related genes. Another approach is utilising gene expression data that is based on the effect of the compounds on gene expression that might either mimic the effect of dietary restriction, long-lived mutants, germ line expression (which is said to be immortal or have rejuvenating effect) or reverse age-related, progeroid or senescent cell gene expression. Another method may be based on a higher level of abstraction where those compounds would have shown previously to reverse a specific age-related change (i.e. not only on the gene expression level).

As anti-ageing drugs interfere with the ageing process they are often effective against numerous age-related diseases. Further, the ageing process seems to be a very ancient and evolutionarily conserved process. Drugs that are shown to be effective in slowing down or reversing ageing in multiple model organisms, including rodents, might also be effective in humans. Lifespan experiments in humans are feasible, but not practical. Therefore biomarkers of ageing are necessary that can serve as proxy indicators of the rate of ageing [Biomarkers of Ageing]. Thus drugs can be tested for their efficiency in reversing certain age-related changes or effectiveness in alleviating age-related diseases. Aged individuals are suitable for any such clinical trials in order to test whether those compounds are capable of reversing the ageing process for these individuals as those individuals have already experienced the effects of ageing. To acquire evidence that the rate of ageing can be slowed would require young individuals being given such drugs. Also the possibility to affect premature ageing diseases or cellular senescence would be a good indication. Although premature ageing may be a special condition not indicative of ageing in the general population.

1.6.3 Classes of Anti-Ageing Drugs

With regard to the mechanism of action, anti-ageing drugs might be classified into defined groups broadly based on the type of anti-ageing strategy. This is primarily attributed to their assumed mode of action.

Possible geroprotectors include resveratrol, sirolimus (i.e. rapamycin; and its derivatives), metformin, spermidine, aspirin (including membrane penetrating aspirin), NAD, lipoic acid, ibuprofen, and carnosine, among many others. Many of those compounds can be classified to be belonging to multiple of those classes (not an exhaustive list):

- Antioxidant
- AGE-inhibitor/breaker
- Pharmacoperone
- Hormetin
- Senolytic

- DR mimetic
- Telomerase-Activating Component
- Sirtuin-Activating Compound (STAC)
- Histone Deacetylase Inhibitor (HDACi)

Most of those have been shown to extend the lifespan in at least one of multiple model organisms. For instance, *Drosophila* fed a biotin diet exhibit a 30% increase in lifespan (Smith, et al., 2007). While others have been found to affect specific types of age-related changes. For example long-term treatment with epithalamin in humans decelerates ageing of the cardiovascular system, prevents age-associated impairment of physical endurance, normalizes circadian rhythm of melatonin production and carbohydrate as well as lipid metabolism. Epithalamin significantly lowers mortality in coronary patients, which indicates that it has a geroprotective effect (Korkushko, et al., 2011).

Antioxidants scavenge free radicals. Supplementation with some specific antioxidants failed to extend the lifespan, but certain antioxidants that were specifically targeted to the mitochondria were found to moderately extend lifespan in specific strains of mice (Anisimov, et al., 2011b).

Yet other kinds of compounds were discovered based on their ability to reduce/eliminate specific types of accumulations of harmful aggregates. Glucose and other reducing sugars tend to react nonenzymatically with proteins leading to the generation of advanced glycosylation endproducts (AGEs) and induce AGE-derived protein cross-linking. AGEs build up slowly and accumulate with ageing which contribute to various pathological events and age-related disease (including nephropathy, retinopathy, vasculopathy and neuropathy). AGE inhibitors and breakers are components that inhibit the formation of AGEs or disrupt the preformed AGE protein cross-links, respectively. AGE breakers in particular are a class of drugs that break the bonds of advanced glycation endproducts. The AGE-inhibitor pimagedine and the cross-link breaker ALT-711 have been shown in animal models and preliminary clinical trials to reduce the severity of pathologies of advanced glycosylation (Vasan, et al., 2001).

Pharmacoperones are small pharmacological chaperones that enter cells and serve as a molecular scaffolding in order to fold and route misfolded proteins. For instance, the amyloid-binding dye Thioflavin T can profoundly extend the median (60%) and maximal (43-78%) lifespan of *Caenorhabditis elegans* and therefore slow down ageing (Alavez, et al., 2011).

Many compounds have been identified by a reductionist approach of being able to stimulate or inhibit a certain class of gene/protein product that itself exhibits aging-suppressing (anti-ageing) or gerontogenic (ageing-promoting) effect. Resveratrol was identified based on its ability to stimulate sirtuins (hence the term Sirtuin-Activating Compound; STAC) (Bonkowski & Sinclair, 2016). Metformin activates AMP-activated protein kinase α (AMPK) (Onken & Driscoll, 2010). TA-65 (a.k.a. cycloastragenol) was specifically developed to activate telomerase (Bernardes de Jesus, et al., 2011; Salvador, et al., 2016). Sirolimus (rapamycin) was found to inhibit the target of rapamycin (TOR) (Anisimov, et al., 2011a). Sodium butyrate is a histone deacetylase inhibitor (Vaiserman, et al., 2012).

Other compounds were found to induce certain kind of processes that are known to have rejuvenating/repairing activities. For example, spermidine was found for its ability to stimulate autophagy (Eisenberg, et al., 2009).

Senolytic drugs (senolytics), like dasatinib and quercetin, are drugs that selectively induce death of senescent cells. Dasatinib eliminates senescent human cell progenitors. Quercetin eliminates senescent human endothelial cells and mouse bone marrow stem cells (Quick, 2015).

Anti-cancer drugs selectively kill cancer cells. Some natural compounds, such as curcumin, exhibit cancer-killing properties.

Another class of drugs are those that mimic the effect of dietary restriction (so called dietary restriction or caloric/calorie restriction mimetics) such as alpha-lipoic acid which can mimic or even block the effect of dietary restriction. Alpha-lipoic acid is also an inhibitor of histone deacetylases leading to hyperacetylated state of a range of proteins as does the classical dietary restriction feeding regimen (Merry, et al., 2008).

Mixtures of components are found to be effective in lifespan extension even when the active ingredient is unknown. For instance, cranberry extract (Guha, et al., 2013; Guha, et al., 2014) promotes healthspan and increases the lifespan by 8.2-80.9% in *C. elegans*. Similar different Ilex paraguariensis extracts robustly extend average lifespan to varying degrees (Lima, et al., 2014).

1.6.4 Brute-Force Drug Discovery: Drug Screening

A way of identifying new anti-ageing drugs would be to screen small molecule libraries via lifespan tests in lower model organisms. An issue would be the selection of the correct dose and the test species. Any drug can be toxic if applied at too high a dose, which would mask its anti-ageing effect. Similarly if too low doses are used no significant effect might be detected. It is advantageous to start screening with a model organism that has a short lifespan. One could start screening in one of the simplest eukaryotic organisms such as budding yeast. Assays can be conducted to increase its replicative and/or chronological lifespan. Such compounds identified could be screened in nematode species, which have a lifespan of about 30 days and also in the fruit fly which have a lifespan of 80 days. An important consideration is the amount of labour required to conduct lifetime studies. Ideally robots can be used to conduct the lifespan assays in a high-throughput manner. Promising compounds identified would then be required to be tested in a short-lived mammal, such as a number of mouse strains, to verify its effectiveness in retarding mammalian ageing. In order to get results in a quicker time frame it is suggested to start applying the drug in adult organisms and see to what extent they are able to modify the remaining lifespan. Biomarkers of ageing and health can be used even earlier to detect anti-ageing properties and reverse signs of ageing (i.e. rejuvenate). In humans starting with already old individuals and assessing ageing biomarkers seems to be the most practical way of testing the effect of anti-ageing drugs. Drugs can for instance quickly be tested for the ability to rejuvenate the immune system in already aged humans (Mannick, et al., 2014).

Without performing lifespan assays drugs can be tested for the ability to reverse different classes of age-related changes. Gene expression profiling can be used to check whether those candidate drugs are able to reset gene expression patterns to that of a young organism when applied to old ones (Merry, et al., 2008).

1.6.5 Targeted Drug Discovery

Targets for small molecule interventions can be identified using bioinformatics. For instance proteins in pathways containing the greatest numbers of genes responsible for lifespan control are attractive targets (e.g. via guilt-by-association).

Modern structure-based drug discovery then enables the identification of novel compounds readily testable in *in vitro* models of age-related diseases in experiments that measure lifespan in multiple organisms. An initial screen can be conducted via genetic means, such as with knockout/overexpression libraries or via gene silencing with whole organisms or just single cells (cell culture). The identified target genes can be used to test drugs that are known to specifically act on those or new drugs can be developed that are likely to enhance or inhibit those gene products.

1.6.6 Pitfalls

There are several pitfalls that need to be taken into account for developing effective anti-ageing drugs:

- Interventions that target "private" mechanisms of ageing
- Involuntary DR
- Hormetic response
- Dose
- Drug-drug interactions

Some common pitfalls in drug discovery for anti-ageing are that the compound may only extend the lifespan in a single type of model organism as it affects a private mechanism of ageing only operating in this model organism type. Therefore validation in multiple organisms is a good indication that it acts via a shared mechanism across phyla.

Some pharmaceutical interventions may provoke DR simply due to suppression of appetite or reduction of nutrient intake and therefore indirectly lead to lifespan extension but primarily via DR (Masoro, 2007). For this reason when testing for the lifespan effect of a compound it is useful to measure the food consumption and compare to controls in order to rule out lifespan extension by sole involuntary DR.

Another problem is hormesis. Even toxic substances might lead to lifespan extension by stimulating repair systems. Whether the hormetic response is additive is rather questionable. It may be so if different *hormetins* stimulate different repair systems.

A further and important issue is the one regarding the dose. Identification of the ideal dose of a drug requires the assessment of a dose response curve by testing different concentrations of the compound (Paracelsus, 1965).

Lastly, the combination of many drugs might need to consider network effects as possible unwanted drug-drug interaction may occur and different drugs may cancel each other out. Ideally would be the identification of drugs that are synergistic with each other and do not cause any unwanted side effects when combined together. The transition from reductionism to a systems-oriented perspective and utilization of systematic approaches in modern biogerontology may result in the development of ageing modulating treatments (Vaiserman, 2014).

Developing anti-ageing drugs is possible and harbours a huge potential. There are different ways of developing drugs against ageing. There are also different types of drugs that can be found to slow down and reverse certain classes of age-related changes. Defined pitfalls need to be paid attention to discover genuine powerful anti-ageing drugs. Testing in multiple model organisms and the assessment of biomarkers of ageing are recommended. Because of the huge search space (number of components and tests to perform) computational approaches are desirable to narrow down the number of potential candidates and needed experiments in order to speed up effective drug identification.

–

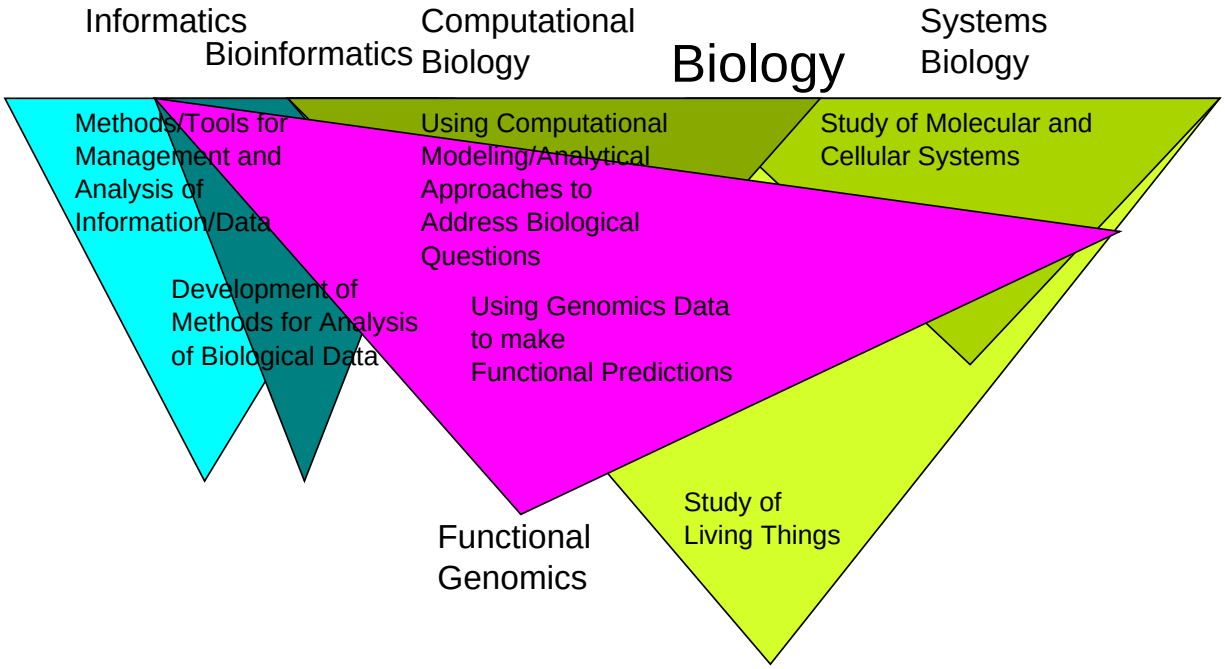

***Figure 1: Overlapping Fields**. Disciplines that are on the intersection of informatics and biology have similar but also different focus and scope as well as utilize overlapping methodologies. Fields are depicted as triangles and laid out in an oversimplified way to illustrate their overlap which is not true to scale for illustrative purpose only.*

1.7 Computational Approaches

Various disciplines utilizing computational approaches that lay on the intersection of informatics and biology are overlapping fields, through each discipline has a different focus as simplified illustrated below [Figure 1 Overlapping Fields] and differentiated in this section in the following.

1.8 Computational Biology

Computational biology is at the interface of computer science and biology. It involves the development and application of data analytics and theoretical methods, mathematical modelling and computational simulation techniques to the study of biological, behavioural, and social systems (Huerta, 2000). Computational biology is the application of core technologies of computer science (e.g. algorithms, databases, artificial intelligence, etc.) to the problems arising from biology. Computational biology builds theoretical models of biological systems, just as mathematical biology does with mathematical models.

Molecular biology is now a high-performance and high-throughput science. One now deals with tera- and petabyte amounts of data. This huge amount of data also takes a lot of computing time. Typically it requires computer clusters.

With the large-scale generation and integration of various omics such as genomics, transcriptomics, proteomics, metabolomics, and interactomics, complex biological systems (which are just machines) and processes could be reverse engineered from data.

Computational biology is particularly exciting today because the problems are large enough to motivate efficient algorithms and the demand of biology on computational science is increasing. The problems are also accessible and are expected to yield medical advances. Overall biology, and molecular biology especially, is increasingly becoming an information science.

Developments in biology are coming astonishingly quickly, generating remarkable possibilities. Computational biology is increasingly of interest in both life science and computational science departments. Many solutions to difficult problems go from biology to computer science: e.g. fragment assembly, sequence analysis, algorithms for phylogenetic trees, evolutionary algorithms and neural networks. In reverse many possible solutions to difficult problems go from computer science to biology: e.g. sequencing by hybridization, DNA computing, etc.

The goal of computational and systems biology is to apply large-scale numerical methods to the study of molecular, cellular and structural biology. The release of the human genome sequence has focused attention on the increasing importance of computational and systems biology for the analysis of gene function. However, only a small fraction of the information generated in modern biology laboratories has been subjected to systematic computational analysis. Thus, the future of systems biology lies not only in improved methods to study sequence information but also in the development of entirely new approaches to the numerical analysis of proteins, cells and organisms.

The cost for DNA sequencing is decreasing at an exponentially pace. The development of ever new techniques of sequencing that power this trend have been termed Next Generation Sequencing. Usually reference genomes are assembled from millions of short reads or nowadays even single molecule sequencing via nanopores (pores of nanometer size) is possible (Maitra, et al., 2012). Genome-wide association study (GWAS) analysis can identify human variants associated with disease. RNA-seq reveals both RNA expression levels and isoforms (transcript variants, i.e. splice variants). Chromatin immunoprecipitation followed by sequencing, or ChIP-seq for short, reveals where key genomic regulators bind to the genome. Quantitative Trait Loci (QTLs) can predict phenotypes. Chromatin accessibility changes can reveal genome functional elements.

One in over 1000 base pairs is different from individual to individual. A fundamental question is which location in the genome has variants that are highly associated to a specific trait or disease and how is that difference causing certain phenotypes?

Computational biology develops large scale computable models for biological research. It uses large scale data from different omics and bioinformatics in order to conceptualize the raw data.

1.9 Bioinformatics

Biological informatics (abbreviated bioinformatics) is an interdisciplinary field that develops methods and software tools for understanding biological data. As an interdisciplinary field of science, bioinformatics combines computer science, statistics, mathematics, and engineering to study and process biological data. Bioinformatics is the research, development, or application of computational tools and approaches for expanding the use of biological, medical, behavioural or health data, including those tools required to acquire, store, organize, archive, analyse, and visualize such data (Huerta, et al., 2000).

It is impossible to do much with this gigantic volume of data unless one uses computer tools to decipher and to find meaningful patterns in the data. Bioinformatics essentially is using computers to analyse a wide variety of biological problems. Bioinformatics is the transformation of data into knowledge and understanding in the area of biology. Bioinformatics started as a small and obscure discipline. Today it is a huge field that is making a digital revolution in biology.

Bioinformatics and computational biology have similar aims and approaches, but they differ in scope where bioinformatics is mostly more general and computational biology problems are often more specific in their focus. Bioinformatics organizes and analysis basic biological data, while computational biology builds theoretical models of biological systems. To be more precise, bioinformatics usually deals with genomics and other omics while computational biology is totally focused on building accurate computational simulations. The aims of bioinformatics are three-fold:

1. Storage: At its simplest bioinformatics organises data in a way that allows researchers to access existing information and to submit new entries as they are produced.
2. Analysis: Further bioinformatics aims to develop tools and resources that aid in the analysis of data.
3. Interpretation: The third aim is to use these tools to interpret the results in a biologically meaningful manner.

Today bioinformatics aims to conduct global analysis of all the available data with the aim of uncovering common principles that apply across many systems and highlight novel features. The computational goals of bioinformatics are:

- Learn & Generalize: Discover conserved patterns (models) of sequences, structures, interactions, metabolism and chemistry from well-studied examples.
- Prediction: Infer function or structure of newly sequences, genes, genomes, proteins or proteomes from these generalizations.
- Organize & Integrate: Develop a systematic and genomic approach to molecular interactions, metabolism, cell signalling, gene expression, etc.
- Simulate: Model gene expression, gene regulation, protein folding, protein-protein interaction, protein-ligand binding, catalytic function, metabolism, etc.
- Engineer: Construct novel organisms, functions or regulations of genes and proteins *in silico*.
- Manipulation: Target specific genes via *in silico* mutation/knockdown to change phenotype.

The central paradigm of molecular biology is:

```
DNA -> RNA -> Protein -> Phenotype (Symptoms)
```

The corresponding central paradigm of bioinformatics is:

```
Genetic information -> Molecular structure
                          -> Biochemical function -> Phenotype (Symptoms)
```

Bioinformatics is a fast-growing interdisciplinary field. As a result of the rising research effort in bioinformatics, the global bioinformatics market was valued at 4,110.6 million USD in 2014 and is expected to reach 12,542.4 million USD in 2020 (Hare, 2014).

Genomic sequencing capabilities have increased exponentially (Lander, et al., 2001; Venter, et al., 2001; Kircher & Kelso, 2010), outstripping advances in computing power (Kahn, 2011; Gross, 2011; Huttenhower & Hofmann, 2010; Schatz, et al., 2010; Cloud, 2012). The rate of available genomic data is increasing approximately tenfold every year, a rate much faster than Moore's Law for computational processing power (Kahn, 2011).

Human genomes differ on average by only 0.1% (Venter, et al., 2001). One thousand human genomes contain less than twice the unique information of one genome. Thus, although individual genomes are not very compressible, collections of related genomes are extremely compressible (Christley, et al., 2009; Brandon, et al., 2009; Maekinen, et al., 2009; Kozanitis, et al., 2010).

Compressive algorithms for genomics have the advantage of becoming proportionally faster with the size of the available data (Loh, et al., 2012). As computing moves toward distributed and multiprocessor architectures, this ability must be considered for new algorithms to be run in parallel.

Bioinformatics is very useful as it forms a basic for other disciplines at the intersection of biology and computer science, as it deals with the raw data and provides algorithms to operate on it, and gathers meaningful insights into biological phenomena. It is, for instance, utilized heavily in functional genomics to infer the functions of biological entities encoded in the genome.

1.10 Functional Genomics

Life comes with information. All information for an organism is encoded in its genome. Functional genomics is molecular biology that utilizes the vast wealth of data produced by genomics (and other omics) to describe the functions of genes and their gene products (including proteins) as well as their interactions. Functional genomic approaches involve the use of large-scale and/or high-throughput methods to understand the function, of major biomolecules, and infer functions from the genome to the phenotype of an organism. In contrast to genomics, functional genomics has a focus on the dynamic aspects such as transcription, translation, gene expression regulation and interactions among biological entities like proteins, as opposed to the more static aspects of genomic information that include DNA sequence and structures. Functional genomics seeks answers to questions about the function of DNA at the level of genes, transcripts and protein products. The key characteristic of studies involving functional genomics is their genome-wide approach to answer those questions, usually involving high-throughput methods rather than reductionistic "gene-by-gene" approach.

1.10.1 Aims

The aim of functional genomics is to generate an understanding of biology in order to bridge the gap between an organism's genome and its expressed phenotype.

Functional genomics is frequently used to refer to the many possible approaches to understand the properties and activities of an organism's genes and gene products. It involves the study of natural variation in genes, RNA and proteins over time as well as both natural or experimental functional interruptions affecting genes and their gene products.

Overall it synthesizes data from various omics (genomics, transcriptomics, proteomics, metabolomics, and interactomics) into an understanding of the dynamic properties of organismal processes and activities. It provides insights into how biological function arises from information encoded in an organism's genome. As the name suggests functional genomics investigates functional-related aspects of the genome such as analysis of mutations and polymorphisms as well as the measurement of molecular activities.

1.10.2 Techniques/Applications

Functional genomics relies heavily on bioinformatics to make sense of the vast amount of data being generated. Among the techniques utilized are data clustering or dimensional reduction (e.g. principal component analysis) for unsupervised machine learning (class detection). Also used are support vector machines and artificial neural networks for supervised machine learning (class prediction, classification). Functional enrichment analysis with ontologies is used for instance to determine the extent of over- or under-expression of genes.

The new generation of sequencing technologies provides unprecedented opportunities for high-throughput functional genomics research. These technologies can be applied in a variety of contexts, including whole-genome sequencing, targeted resequencing, discovery of transcription factor binding sites, and coding/non-coding RNA expression profiles as well as epigenetic marks (Morozova & Marra, 2008).

1.10.2.1 Genome

Systematic pairwise deletion/overexpression of genes or inhibition/activation of gene expression can be utilized to identify genes with related function, even if they do not interact physically.

The ENCODE (Encyclopedia of DNA Elements) project builds a comprehensive parts list of functional elements in the human genome, including elements that act at the RNA and protein levels and regulatory elements that control cells and circumstances in which a gene is active. It is intended as follow-up to the Human Genome Project to map all functional elements in the human genome.

1.10.2.2 Epigenome

Bisulfite sequencing is the utilization of bisulfite treatment of DNA to determine its pattern of modifications such as methylation and hydroxymethylation.

ChIP-sequencing (ChIP-seq) is a technique to analyse protein interactions with DNA. It combines chromatin immunoprecipitation with massively parallel DNA sequencing to identify the binding sites of proteins associated with DNA.

1.10.2.3 Transcriptome

Microarrays measure the amount of RNA in a sample that corresponds to a probe DNA sequence or gene.

An alternative method of gene expression analysis is SAGE (serial analysis of gene expression) that is based on RNA sequencing rather than hybridization.

1.10.2.4 Proteome

A yeast two-hybrid (Y2H) screen assesses a "bait" protein against many candidate interacting proteins ("prey") to identify physical protein-protein interactions.

Affinity purification and mass spectrometry (AP/MS) is capable of identifying proteins that interact with one another in complexes.

1.10.2.5 Loss/Gain of Functions

Mutagenesis is commonly used to alter genes. The function of genes can be investigated by systematically "knocking out" or "overexpressing" genes one by one. This is done either by disruption of function (via deletion or insertional mutagenesis) or by enhancing function (via inserting multiple copies or rendering promoters more active).

RNAi (RNA interference) is a natural biological process in which RNA inhibits gene expression or translation by neutralizing a target mRNA. This process can be used to transiently knock-down the expression of a gene of interest using targeted short double-stranded RNA. The knock-down can also be made permanent if DNA expressing interfering RNA is introduced into the cells.

Functional genomics uses genomics data to study gene and protein expression and function on a global scale (genome-wide or system-wide), focusing on gene transcription, translation and protein-protein interactions, and often involving high-throughput methods. Overall, functional genomics has the goal to understand the relationship between an organism's genome and its phenotype. Functional genomics helps to lay a solid foundation for the systems biology approach.

1.11 Systems Biology

Systems biology is an approach in which *experimental biology is closely integrated with mathematical or computational modelling* in a synergistic way to answer biological questions that would not be possible by empirical approaches alone. One of the goals of systems biology is to discover new emergent properties that may arise from studying the system as a whole, leading to more rapid and deeper understanding of how the system is controlled and how it responds to external stimuli. This level of understanding will greatly facilitate the future of biological systems.

Systems biology aims to develop hypotheses based on integrated or modelled data. A systems biology project may be performed at a number of different biological scales or it may integrate across scales. The systems biology cycle is composed of modelling and experimentation. Models should be both descriptive and predictive (Antezana, et al., 2013). Systems biology provides an understanding of biology from a system perspective, moving from components and interactions into groups or sets of components that are associated with a function and how these subcellular functions give rise to cell- and tissue-level functions.

There has been a vast amount of knowledge that was gained. This knowledge allows now to gain a perspective where one can start at the level of genes, and go to the level of the cell, tissue and organismal level and understand how the information in genes is decoded to form proteins and how proteins interact with the subsets of lipids and sugars and so on. All of these together give rise to cellular tissue and organismal function. It is this kind of integrated study that is called systems biology.

The word system itself is an amorphous term. It can mean systems at various levels of biological organization. Some might have a system at the level of a cell or the level of a tissue or an organ or the level of the whole organisms, or going back to the other end, it may be subcellular levels, either mitochondria or the nucleus can also be called systems. So there is not one fixed definition of what systems biology will be and different people can have different perspectives of how the field is growing and focused on.

Focusing largely on systems biology at the cellular level, and mostly mammalian cells, provides a natural entry into how cells become tissues and organs, and allow systems approaches to the study in medicine, pharmacology, and therapeutics. Systems biology requires a sense of how quantitative reasoning can be used to deal with large datasets and an understanding of what kind of mathematical representation is appropriate for different kinds of biological questions and systems. Mathematical analysis can provide a deep understanding of how behaviours occur and emerge, and how one can get predictive value from computational analysis of for instance high-throughput data generated by molecular profiling.

1.12 Molecular Profiling to Decipher Ageing

1.12.1 Background

A molecular profile can be defined as a snapshot of a defined biological state. Such a profile can be a representation of some property of different types of biological entities (RNAs, proteins, metabolites, etc.) and it can be an explicit determination (exact numbers/concentrations) or a relative examination (e.g. intensities).

A molecular signature in contrast is the result of comparison of two states such as old vs. young, diseased vs. healthy and mutant vs. wild type. High-throughput techniques, in particular transcriptomics, have been

widely used in recent years to generate genome-wide profiles associated with disease states or particular processes (de Magalhaes, et al., 2009a). A widespread problem, however, is the difficulty to distinguish cause from effect, to uncover which molecular changes reflected in a profile are the most important ones, i.e. drivers, and which are non-crucial, i.e. passengers (de Magalhaes & Toussaint, 2004).

–

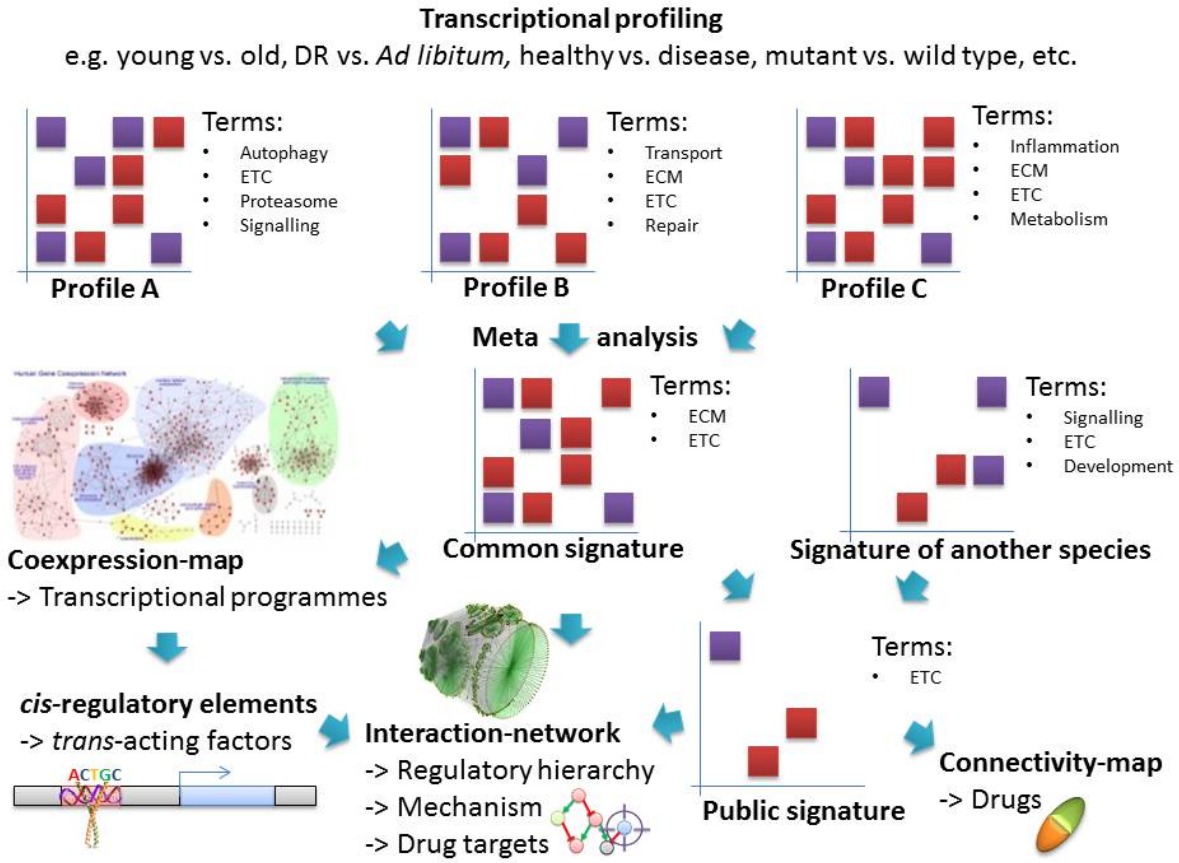

Figure 2: Transcriptional Profiling for Drug Discovery*: Defined processes or diseases are transcriptional profiled and enriched processes (i.e., functions common to multiple genes) are identified. Common signatures can then be generated by meta-analysis of multiple individual experiments. A signature is composed of different transcriptional programmes, which can be dissected by follow-up transcriptional analyses. Interaction networks focus on the underlying hierarchy in gene regulation and the mechanistic basis of the process under study, which in turn enables the identification of the most suitable drug targets and regulatory genes. A signature can be used for pattern matching in drug databases in order to identify drugs which induce or counteract this gene expression pattern.*

Comparative analysis of molecular profiles (typically gene expression) can identify highly robust patterns that occur most consistently among multiple experiments. A signature can therefore be defined as a common pattern, like a fingerprint, reappearing in the comparison of multiple profiles. Because they incorporate multiple profiles, often from disparate experiments, signatures have improved statistical power and are hence more reliable. Comparing multiple profiles can uncover which signatures are shared and which are unique, for instance in different tissues, different species or across pathological states. In the context of the ageing process it is of particular interest, for instance, to identify signatures common across tissues or even between species (public mechanisms) as well as shared by multiple lifespan-extending interventions.

Transcriptomics with DNA microarrays and RNA-seq have generated a huge amount of molecular profiles. These can be integrated, reanalysed and interpreted in context of biological significance to generate

signatures, identify pathways that may underpin a given process or disease, identify biomarkers, and thus derive new causal links and even identify new targets for drug discovery [Figure 2 Transcriptional Profiling for Drug Discovery].

1.12.2 Methodologies

There are various statistical methods to analyse microarray data to determine which genes are differentially expressed across two groups of samples in order to derive transcriptional signatures. Among those methods are the t-test, regression modelling approach and mixed model approach as well as empirical Bayesian method and Significance Analysis of Microarray (SAM), limma and many others (Pan, 2002). Because of this complication it is wise to assemble profiles in such a way that it is flexible and easy to derive signatures via these different methods or any other future statistical approaches. Such an approach would identify which methodological approach was superior.

The identification of differentially expressed genes (i.e. derivation of molecular signatures) in experiments with small sample size, high dimensionality, and high variance remains extremely challenging, which limits the usability of tens of thousands of publicly available, and possibly many more unpublished gene expression profiles (Vasiliu, et al., 2015). A variable selection algorithm for ultra-low sample microarray studies using generalized linear model-based variable selection with a penalized binomial regression algorithm (penalized Euclidean distance) has been proposed (Vasiliu, et al., 2015).

For deriving a signature from molecular profiles in the context of ageing, usually individuals are sorted into four distinct age bins (developing, young, middle-aged, and old) and age-related genes are identified by comparing each group to the consecutive one (Berchtold, et al., 2008). Modelling age-related gene expression data using regression models can increase the statistical power to identify gradual changes across lifespan (Yuan, et al., 2012). Logistic regression-based approach in combination with an intensity-based Bayesian moderated t-test was used to identify sex- and ageing-related functional groups of differentially expressed transcripts (Liu, et al., 2013). A regression-based differential expression detection algorithm can be used for profiling studies with ultra-low sample size (Vasiliu, et al., 2015).

Different comparisons of gene signatures were implemented. A non-parametric methodology can be used to detect statistical significance of overlaps of ranked lists of genes and estimate the number of genes with a common expression profile. This has been used to build a conserved health span signature and found to depend on tissue type (Antosh, et al., 2011a).

Gene-set enrichment analysis is a statistical functional enrichment analysis commonly applied to identify enrichment of biological functional categories in sets of ranked differentially expressed genes from genome-wide expression profiles (Clark & Ma ayan, 2011). An alternative approach of deriving consensus signatures and calculating the similarity of signatures would be based on gene sets, rather than individual genes. While individual genes might be different across various signatures of a defined phenomenon, those signatures might regulate the very same process in the same way but just act on, or by different genes.

Transcription factors that are responsible for the observed transcriptional signatures can be identified either via target gene collection or consensus motif scanning of the regulatory regions of the differentially expressed genes within the signature. Transcription factor knockout and overexpression signatures as well as transcription factor binding signatures can be used for this purpose as well. While a comparative analysis of transcription factor regulation inferred from mutant experiments is possible, functional assays are required for accurate identification of transcription factor binding site interactions (Kahana, et al., 2010).

Meta-analysis is used to derive signature from published data. It is possible to perform meta-analysis of transcriptomics studies with arbitrary experimental designs by deriving global expression features rather than decomposing studies into multiple phenotypes comparisons (Caldas & Vinga, 2014).

1.12.3 Types of Signatures

Molecular signatures are an important and flexible concept capable of providing significant insight concerning the underlying process when analysed from high and low-throughput omics data. High-throughput 'omics' technologies such as genomics, epigenomics, transcriptomics, proteomics, metabolomics and metagenomics are enabling detailed investigations of molecular changes and are thus revealing information about biological processes, activities and locations that change with age.

Molecular signatures can be classified based on the type of molecular entity they represent as well as sources they were derived from:

Molecular Entity:

- Genomic Signature
- Epigenomic Signature (Epigenetic Signature)
- Transcriptomic Signature (Transcriptional Signature)
- Proteomic Signature
- Metabolic Signature

Source:

- Single Source Signature (i.e. same platform and same laboratory, etc.)
- Consensus Signature (Combined Signatures)
    - Tissue-Specific Consensus Signature
    - Cross-Tissue Consensus Signature (Multiple Tissue Common Signatures)
    - Cross-Species Consensus Signature (Multiple Species Common Signatures)

Global gene expression analysis utilizing microarrays or RNA-seq allows to understand biological processes at a system level. With the parallel increase in generated data, and in precision and coverage of this technology, reconstruction of molecular mechanisms from data becomes more feasible (Vasiliu, et al., 2015). Instead of focusing on one or even several pathways in studying the molecular processes of ageing, an increasingly powerful approach has been the global screen of the entire genome for age-associated genes and genetic pathways. Microarrays and RNA-seq can be used to screen the transcriptome and with this basically the genome of several organisms, for genes and pathways that change in expression levels with age or under conditions that counteract ageing (Zahn & Kim, 2007). DNA microarrays and RNA-seq enable the generation of transcriptional profiles for the ageing process and interventions that interfere with it. It is a very promising approach for identifying biomarkers of ageing.

Other omics data that can be used to derive molecular signatures and which is becoming more plentiful are epigenetic profiles such as DNA methylations, proteomic profiles and metabolomic profiles. Genetic signatures can be established from genome-wide linkage and genome-wide association studies (GWAS). Epigenetic signatures can be created for DNA methylation and DNA hydroxymethylation as well as from histone occupation and histone modification profiles for various post-translational modifications of histone tail residues. Epigenetic signatures can be derived from bisulfite sequencing (DNA methylation and DNA hydroxymethylation) as well as ChIP-chip and ChIP-seq against histones and their modifications as well as chromatin remodelling binding patterns. Distribution of DNA-binding molecules and histone modifications have been profiled in models organisms such as the fruit fly (Schwartz, et al., 2006) and mice as well in various human cell lines and tissues, which provide valuable resources to derive further kinds of molecular signatures. It would be to interesting to use it for gene-set analysis as well. Transcriptomic signatures are generated mainly from DNA microarrays and RNA-seq. Most transcriptional profiling do not provide information on non-coding RNA such as microRNA, for which special arrays are usually employed. Proteomics and metabolic signatures can be derived from mass spectrometry.

While transcriptional signatures give significant insight into the underlying processes they represent on a genome-scale level, the restriction onto transcript levels only would be insufficient to detect subtle changes that are not reflected in the transcriptome. It would be informative to also derive proteomics signatures.

However, even proteomics signatures have some limitations. The overall abundance of some proteins remains unchanged during ageing but subcellular location, post-translational modification state, or splice form varies (Ori, et al., 2015). Some protein-level differences appear to be a generic property of age, the majority are specific to one organ. They may be the consequence of the physiology of the organ or age of the cells within the tissue (Ori, et al., 2015).

A major challenge is the genome-wide integration of different omics such as transcriptome and epigenome (Natsume-Kitatani, et al., 2011). Combining microarray expression profiles with other data sources such as motif and ChIP-chip data enables insight into the transcriptional regulation that leads to certain phenotypes such as ageing or lifespan extension (Cheng, et al., 2007). Genetic, epigenetic, transcriptomic and proteomic signatures could be combined as all of these could be mapped mostly to genes. Alternatively, signatures of heterogeneous entities composed of genes, transcripts, proteins, and metabolites could be established, but their processing and analysis is non-trivial.

1.12.4 Opportunities

Molecular signatures can be used to derive biomarkers of ageing and interventions such as dietary restriction. Genes that are down-regulated during ageing and up-regulated under lifespan-extending interventions are candidates for anti-ageing targets to be enhanced. Genes that are up-regulated during ageing, but down-regulated under lifespan extension could also be potential targets of suppression in anti-ageing interventions.

A range of omics approaches and different study designs are required to investigate human ageing in order to recognize molecular changes that are relevant to the process. Some of these study designs can assist to establish causalities. Discriminating between cause and effect may require the integration of multi-level omic data with varying phenotypes over time.

1.12.5 Challenges

Most molecular profiling of human gene expression changes were produced by cross-sectional studies, i.e. a molecular measure is obtained in a group of individuals of different ages. These studies estimate changes with age from the differences between individuals of different ages and the results may be confounded by unknown inter-individual differences, therefore resulting in inaccurate estimates of age-related changes. In model organisms this issue is not so severe as such studies usually utilize genetically identical individuals and can follow the same individuals throughout the life (longitudinal study).

Another important issue is that molecular profiles are analysed with respect to chronological age, which may not reflect the biological age of the individuals profiled in cross-sectional study designs. Individuals have different ageing rates, which should be taking into account when grouping individuals or applying mathematical models.

Sometimes raw data is not available and eventually only significant genes in an article or supplementary material are presented if at all. One could try to contact authors to request the raw data, but that is very cumbersome and might not be so fruitful. Preferentially one could be able to integrate at least those results reported in the article or supporting material if no raw data is obtainable. Moreover, ideally one could be able to combine high-throughput with low-throughput experiments all together.

Gene expression profiling studies should always incorporate age as a parameter even if studying a specific disease whenever using samples from individuals with different age. Also time of day should be considered if possible in order to account for circadian changes in gene expression.

1.12.6 Tissue-Specific Signatures

Complex multicellular organisms such as humans have different types of tissues, with very different functionality and patterns of gene expression and therefore the process of ageing might manifests itself in different ways in different tissues (Arking, 2006). However there are only a few basic types of tissues such as epithelial, connective, muscular, and nervous tissues (Kinne-Saffran & Kinne, 1994). Basic tissue-specific signatures therefore, might be appropriate. There are various subtypes of tissues within each of these primary tissues.

Tissues are composed of cells. The body has many different kinds of cells. A tissue-specific signature is therefore usually an average of the gene expression patterns of a mixture of cells in this tissue. Although their appearance might be very different, most cells have chemical and structural features in common. In humans, there are around 200 different types of cells, and within these cells there are over 20 different types of structures and organelles. The cellular composition of tissue may change and changes detected in molecular signatures might merely represent the changes in the abundance of cell types rather than changes in the gene expression patterns of the cells within the tissue. This means that the cell type abundance can change, i.e. some cell types become more abundant while others become less and this might be reflected in the expression signature. Signatures that characterize a cell type might be useful to differentiate the effect of cell population changes within a tissue.

In aged mammalian organisms there is a mixture of senescence and non-senescence cells. A signature of an old tissue is therefore reflecting the average expression levels in non-senescence and senescence cells. There are many different ways how cellular senescence can be induced. Although the state of senescence established by these different triggers is very similar and the phenotype almost identical, there are also subtle differences which maybe important differences. A signature representing senescence would be useful, but also senescence type-specific signatures (e.g. replicative senescence signature, oncogene-induced senescence signature, etc.) might be required as well as senescence cell type-specific signatures. Because of the high number of signatures that could be established, a consensus signature seems to be most practical and intuitive.

With the advent of single-cell sequencing, cell-type specific signatures could be established. This could help to determine the changes in defined cell types and the change in abundance of certain cell types as well as in senescence cells.

Gene expression changes with age are usually assumed to be tissue-specific (Zahn, et al., 2006). Similarly the response to dietary restriction is often associated with tissue-specific changes. Tissue-specific molecular signatures of ageing do not only correlate with chronological age but also with measures of physiological age (Zahn, et al., 2006).

Similarly as with gene expression changes with age, DNA methylation changes have been assumed to be different from tissue to tissue. However ageing-differentially methylated regions in one tissue have been replicated in totally different tissues (Rakyan, et al., 2010).

1.12.7 Gender-Specific Signatures

The relative importance of gender-specific age-related changes is not yet certain. Whether there are common age-related changes between male and female organisms is an open question. There is evidence supporting the importance of gender-specific changes but also evidence supporting gender-common changes. For instance in human skin age-related changes in gene expression seem to be sex-specific (Swindell, et al., 2012).

Some transcript levels are changing in the opposite direction during ageing in males and females (Liu, et al., 2013). So these cases might be considered to be sex-specific signatures. Further, there is overall evidence that females age slower than males and therefore females life is usually longer than males (which is maybe true in humans, but it is not always the case in different species). This is observed in humans as well as in many model organisms. This means that it would maybe be better to not pool male and females while deriving molecular signatures of ageing as females tend to age slower and therefore similar chronological aged females might actually be biological much younger than their male counterparts.

1.12.8 Tissue-Specific Consensus Signatures

Several attempts have been made to create consensus signatures of normal and accelerated ageing as well as dietary restriction. A variety of approaches have been tried to derive tissue-specific as well as common consensus signatures. For example AGEMAP project catalogues the changes in gene expression as a function of age in 16 tissues in mice (Zahn, et al., 2007).

1.12.8.1 Blood

Datasets of genome-wide gene expression levels in human peripheral blood mononuclear cells were compared for the effect of ageing and chronic social stress. It was found that the direction, although not the magnitude of the significant gene expression changes tends to be shared between the datasets at the level of individual genes as well as gene functional categories of genes and ageing implicated molecular pathways (Snyder-Mackler, et al., 2014).

In an integrative network-based approach, multiple large-scale expression studies in human blood were combined with molecular interaction data to detect consistently coexpressed interaction modules that may reflect the change throughout ageing (van den Akker, et al., 2014). This meta-analysis of blood transcriptome identified coexpressed protein-protein interaction modules as robust biomarkers of (chronological) ageing in humans (van den Akker, et al., 2014).

1.12.8.2 Skin

The ageing of skin is associated with processes that compromise the structure of the extracellular matrix and promote loss of functional regenerative capacity. Mouse and human skin ageing were compared at the level of the transcriptome and the overall correspondence was found to be weak. Ageing does not uniformly heighten inflammatory status across all tissues (Swindell, et al., 2012).

The ageing process in human skin is connected with deregulation of numerous cellular processes such as cell cycle control, cytoskeletal changes, inflammatory responses, signalling and metabolism (Lener, et al., 2006). In the derivation of a human skin ageing signature, the young group was defined too young: 3-4 years corresponds in humans to developing (not yet fully matured) individuals, while 68-72 years corresponds to old adults.

1.12.8.3 Breast

Transcriptomic profiling of breast ageing and cancer were performed in combination with network analyses to identify age-specific signatures. Using cross-species comparative genomics approach molecular alterations during cancer progression in young and old females were studied. Differential expressed genes were found to be associated to immune response, tissue morphology, cellular growth and proliferation, cell death and cellular movement (Colak, et al., 2013).

Meta-analysis of female breast tissue leads to the generation of breast-specific consensus signature of ageing. It was found that epigenetic transcriptome changes, more than genotype variation, account for age-associated differences in sporadic breast cancer (Yau, et al., 2007).

1.12.8.4 Heart

The heart of humans is capable of functioning for decades despite minimal cell turnover or regeneration (Kaushik, et al., 2015).

Transcriptome data was integrated from multiple cardiac-specific transcriptional profiles derived from independent studies in mouse and human together with proteome and micronome (interactome of microRNA transcripts) interaction data by deriving multiple independent weighted networks. Through modularization of those weighted networks generated modules that were redefined to form consensus modules across datasets that change substantially during lifespan. Such modules were proposed as signatures (Dimitrakopoulou, et al., 2015).

1.12.8.5 Brain

A tissue specific meta-analysis for brain has been generated. It was found that Alzheimer's disease and frontotemporal lobar degeneration exhibit a significant difference between physiological and chronological ages and hence exhibit prematurely aged expression profiles (Cao, et al., 2010).

Significant differentially expressed genes across age in male rats (Fischer 344) were identified by meta-analysing hippocampus gene expression data that may explain memory impairment with old age (Uddin & Singh, 2013).

Gene expression with age in the human male and female superior frontal gyrus (SFG), part of the prefrontal cortex; the postcentral gyrus (PCG), part of the somatosensory cortex; the hippocampus (HC) were subjected to meta-analysis. Polynomial regression was used for identifying age-related gene expression changes. It was found that age-related gene expression changes occur earlier, or at a faster pace in females compared to males. Thus this may indicate that those brain regions age faster in females, which may explain why females tend to get Alzheimer's disease earlier than males (Yuan, et al., 2012).

Meta-analysis of brain ageing and neurodegeneration quantified the similarity and differences in genome-wide gene expression profiles using non-linear regression. It applied a computational method for assessing an individual's physiological age via the comparison of global expression profiles across a range of normal human brain samples and found that neurodegenerative disease tissues exhibit prematurely aged expression profiles (Cao, et al., 2013).

Prefrontal cortex (brain) of human and rhesus monkey over developmental and ageing intervals of their respective lifespans were profiled for mRNA changes via RNA-seq and protein expression change as well as human brain ageing meta-analysed. A substantial decoupling of mRNA and protein expression levels was found during ageing, but not development (Wei, et al., 2015)

Integrated miRNA and mRNA expression profiles of human brain across lifespan (i.e. development and ageing) were meta-analysed and age-related miRNA were detected via polynomial regression models. A miRNA-mRNA regulatory network was constructed by pairwise correlation coefficient analysis between miRNA and mRNA expression profiles. Age-related miRNA models were identified (Li, et al., 2013)

Age-dependent differential expression was detected in human prefrontal/frontal cortex ageing in a meta-analysis via fitting quantile regression (constant, linear and piecewise linear) models to the expression profile of each gene (and selected the least complex model that fits the data best) to capture linear and non-linear changes. In addition, age-differential variability was identified by fitting and comparing two quantile regression models to the expression profile of each gene. Such a model-selection approach might be more robust than standard linear regression to discover age-dependent patterns and is applicable to many ageing and other time-series datasets (Ho, et al., 2009).

Meta-analysis of human frontal cortex ageing gene expression data found gene markers that assembled into a transcriptional module in a gene coexpression network. A subnetwork of transcription factors were found to regulate the age-dependent module. Causal contributions of these master regulators were verified using ChIP-seq data. The interaction of the key master regulators were found to be preserved in protein-protein interaction network. These master regulators seem to be essential for regulating microglia homeostasis in the frontal cortex of adults in addition to their crucial roles in haematopoiesis and myeloid cell-fate decisions during embryogenesis (Wehrspaun, et al., 2015).

Inflammation has often been observed to be upregulated in multiple tissues during ageing. In the neocortex and cerebellum of mice, ageing results in gene expression changes indicative of an inflammatory response, oxidative stress and reduced neurotrophic support and dietary restriction selectively attenuates the age-associated induction of genes encoding inflammatory and stress responses (Lee, et al., 2000). Age-related neurodegenerative disorders are characterized by degeneration of neurons in specific brain regions (Mattson, et al., 2001) that may be caused by the upregulation of inflammation with age and the downregulation of neurotrophic support. The transcriptome of irradiated microglia seems to exhibit ageing-like changes (Li, et al., 2015).

1.12.9 Tissue-Common Consensus Signatures

1.12.9.1 Normal Ageing Signatures

Ageing is characterized by a decline in physiological function in different organs that in turn is associated with altered gene expression. Multiple microarray studies of ageing have been performed with the aim of identifying biomarkers as well as genes and pathways differentially expressed that may give insights into the underlying mechanisms of ageing (de Magalhaes, 2009).

By and large, gene expression changes with age are modest and age-related gene expression profiles generated by different studies often do not overlap (de Magalhaes, et al., 2009a). Different mechanisms may operate in different tissues and even common mechanisms may trigger different responses in

different organs. A few studies, however, identified common signatures across ageing tissues in mammals and invertebrates and these provide clues into conserved mechanisms of ageing.

A transcriptional signature derived from human muscle tissue performed consistently in other non-heart muscle tissue samples, skin and brain tissue. The signature is insensitive to confounding lifestyle biomarkers and diagnostic for health (Sood, et al., 2015).

By meta-analysing large-scale transcriptional changes in ageing from DNA microarray profiles of multiple tissues in mouse (AGEMAP), a modular decline in co-expression with age was found. Specifically, gene co-expression networks for 16 and 24 months old mice were derived and a number of functional gene groups were identified that change co-expression with age. It has to be noted that 16 and 24 months old ages are both classified to be old adults. It was found that within these changing groups there was a trend towards declining correlation with ageing. Specific transcription factors (NF-kappaB, MEF2, RREBP1, AP2 and MZF1) were identified where gene expression correlated with age. Further targets of NF-kappaB transcription factor that decreased expression with age were computationally identified. Genes that are prone to decline in co-expression tend to be co-located on the same chromosome (Southworth, et al., 2009). The loss of coexpression and increased gene expression variability was noted also in other studies.

A method was developed to identify biomarkers of ageing that are networks of genes selected based on age-dependent gene expression changes and the graph-theoretic property of modularity (which measures the strength of division of a network into modules/groups/clusters/communities). This method was applied on *C. elegans* ageing time-series transcriptome data. The modular biomarkers can be used to assign novel ageing-associated functions to uncharacterised ageing genes (Fortney, et al., 2010).

Transcriptional changes during ageing in brain, eye, kidney, muscle, skin, and blood were meta-analysed to create a common signature of ageing and compared to a common signature of cancer (Wang, 2012). Most age-related gene expression changes were found to be tissue-specific and few were found to be common to multiple tissues (Wang, 2012). A large proportion of ageing signatures are universally differentially expressed among tumour phenotypes (Wang, 2012).

Tissue-specific transcriptional ageing signatures were derived (by using the AGEMAP). Coordinated ageing processes across different tissues were identified in cross-tissue coexpression networks built based on both genes and pathways. Thereby a cross-tissue common signature of coordinated ageing across different tissues was established (Huang, et al., 2011).

Gene expression changes with age are usually quite noisy. A meta-analysis tried to identify common consensus signature of ageing across tissues and different mammalian species. For this a common ageing signature in mammals was derived from 27 different studies of transcriptional profiles conducted in mice, rats and humans via a meta-analytic value counting approach (de Magalhaes, et al., 2009a). The conserved signature is composed of discrete transcriptional programmes and revealed an overexpression of immune response and inflammation, lysosome and apoptosis associated genes, while collagen and energy metabolism (especially mitochondrion and oxidative phosphorylation-related genes) were underexpressed with ageing. Genes known to be overexpressed in senescent cells, such as fibronectin, p21, clusterin and apolipoprotein D, were also found commonly overexpressed during ageing in mammalian tissues, hinting that the accumulation of senescent cells does occur *in vivo*. The ageing molecular signature reflected a combination of patterns accompanying degenerative processes (decreased energy metabolism and collagen gene expression) as well as the activation of protective responses (e.g. *APOD*, *CLU*, *MGST1*, lysosomal and detoxification genes). The upregulation of genes and pathways with protective functions suggests they are part of transcriptional responses to ageing. In other words, these may be processes that fend off normal ageing and it is thus possible that upregulating these genes earlier in life will have later life health benefits (de Magalhaes, et al., 2009a).

Specific signatures were identified for ageing, Alzheimer's disease and amyotrophic lateral sclerosis mouse model. The gene expression signature of microglia priming was determined by comparing the transcriptome of microglia in ageing, Alzheimer's disease and amyotrophic lateral sclerosis mouse models using Weighted Gene Co-expression Analysis (Holtman, et al., 2015).

A meta-analysis of neurodegenerative disease profiles identified a common transcriptional signature of neurodegeneration (Li, et al., 2014). Mouse models rarely mimic the transcriptome of human neurodegenerative diseases (Burns, et al., 2015).

Age-related gene expression changes in nine tissues were meta-analysed. Ageing gene expression signatures were found to be very tissue-specific (Yang, et al., 2015).

A derived muscle signature was meta-analysed with human kidney and brain and subsequently with mouse and fly data (Zahn, et al., 2006). Analysing transcript expression of human muscle tissue generated a molecular profile consisting of 250 genes (Zahn, et al., 2006). The signature correlated with chronological age as well as with measures of physiological age. Comparing the transcriptional profiles of muscle with those of kidney and brain revealed a common signature of ageing in these diverse tissues. This common ageing signature consisted of six genetic pathways, of which four pathways increase expression with age (extracellular matrix, cell growth, factors involved in complement activation and components of cytosolic ribosome), while two pathways decrease expression with age (chloride transport and subunits of mitochondrial electron transport chain). The age-related decrease in electron transport chain was also common to the transcriptional profiles in human, mouse, fly and worm (Zahn, et al., 2006). The same authors later systematically analysed 16 tissues from mice of age 1, 6, 16 and 24 months. This study (AGEMAP) revealed that some tissues display considerable transcriptional differences (e.g. thymus) in old mice, while others had few or no changes (e.g. liver and striatum) in expression with age. Interestingly, gene expression changes with age in different tissues correlated with each other in individual mice, suggesting that different tissues age in a coordinated fashion. Cell cycle inhibitors (e.g. p16INK4a) had an increasing trend in expression with age in mice and humans, which may imply a reduced ability of cells to proliferate in old organism (Zahn, et al., 2007).

1.12.9.2 Premature Ageing Signatures

A meta-analysis of a premature ageing syndrome (Hutchinson-Gilford Progeria Syndrome; HGPS) and its comparison to normal ageing in fibroblasts was conducted. Changed signalling of HGPS were found to strongly resemble that from cells of middle-aged and old individuals (Aliper, et al., 2015).

A common transcriptomic signature of premature ageing mouse models was derived. From this profile a strong suppression of thyroid hormone signalling in specific peripheral organs in premature and normal ageing was found (Visser, et al., 2016).

1.12.9.3 Cellular Senescence Signatures

Molecular signatures of human cellular senescence and tissue ageing were compared by utilizing transcriptomics data from human cell- and biopsy-based DNA microarrays studying cellular senescence or *in vivo* ageing, respectively. Certain genes and pathways (such as cancer, Huntington's disease, MAPK signalling, focal adhesion, actin cytoskeleton, oxidative phosphorylation, and metabolic signalling) were found to be co-regulated during cellular senescence and *in vivo* tissue ageing (Voutetakis, et al., 2015).

Replicative senescence is associated with highly reproducible epigenetic modifications. The state of senescence can be monitored by continuous DNA methylation changes at specific genomic sites and therefore provides an epigenetic biomarker to determine the state of senescence in cell preparations (Koch, et al., 2012).

1.12.9.4 Dietary Restriction Signatures

Ageing is surprisingly plastic as it can be manipulated by genetic and environmental factors. One of the most widely studied non-genetic interventions is dietary restriction (DR) of certain factors in the diet, such as calories or certain amino acids, which without malnutrition can robustly extend lifespan and delay the ageing process in several model organisms (Lee, et al., 1999). The exact mechanisms of DR remained poorly understood and so unbiased gene expression studies of DR can provide insights. Early studies of DR using microarrays showed metabolic alterations (Lee, et al., 1999) and many more studies have been conducted to date revealing a complex array of DR-induced changes. Meta-analyses incorporating these multiple studies have the potential to uncover conserved signatures that include signalling pathways responsible for the life-extending effects of CR.

One meta-analysis comparing and contrasting the signature of the ageing (22 tissues) and DR (17 tissues) in mouse showed that DR broadly induces expression of genes regulating oxidative balance, anti-inflammation (e.g. inhibition of NF-KappaB) and cell proliferation, while it suppresses expression of

extracellular matrix components and inflammation. Clearly, DR opposes age-associated expression patterns with respect to gene subsets associated with certain biological processes (e.g. immunity and inflammation). Discrete functional coexpression modules, formed commonly by up- and/or down-regulated genes, were identified, suggesting that DR and age-related gene expression patterns exhibit strong modularity. Among the DR coexpression modules were histone cluster, period (gene) homologs as well as mRNA processing and transcriptional regulation factors (Swindell, 2009).

Another meta-analysis study found that merged gene expression profiles for both ageing and DR from multiple tissues were both enriched for immune response and cell adhesion, while DR attenuated them. Lipid metabolism was suppressed by ageing and becomes more active under DR. Functional modules with consistent expression patterns in a coexpression analysis revealed systemic interactions among biological processes, such as a negative relation between lipid metabolism and immune response. Lipid metabolism was also inversely related to cell cycle, cell proliferation and muscle development, while the immune response exhibited positive correlation with these systems. Transcription factor binding sites of the CCAAT box and forkhead family were shared by ageing and DR profiles (downregulated genes during ageing and upregulated genes upon DR). Further cooperative activities of transcription factors such as *Elk-1* (which was found to regulating significant number of ageing upregulated genes) and CCAAT box might modulate stress response genes during ageing (Hong, et al., 2010).

Intersection of the whole-genome expression profile in response to DR, mutations mimicking DR, heat shock and H2O2 exposure in yeast based on functional terms, indicated that the mitochondrion and the electron transport chain appear to be major hubs (i.e. nodes with high degree, hence many connections) in the gene expression program under these conditions. Genes involved in respiration and transport of metal ions (iron and copper) were up-regulated. Upon DR feeding the levels of iron and copper fall in cells, which elicits a transcriptional response upregulating the genes involved in their uptake to maintain cellular homeostasis (Sharma, et al., 2011).

Acute DR alters mortality rates within days in flies (Selman, et al., 2006a). This implies that profiles of animals put on acute DR should already obtain the necessary changes for lifespan extension relatively quickly.

Gene expression profiles from eight tissues of mice subjected to dietary restriction were analysed and a common transcriptional signature was identified that includes genes associated with mitochondrial energy metabolism, inflammation and ribosomal structure. This signature is detected in flies, rats, and rhesus monkey on dietary restriction. Further, the signature was also detected in mouse genetic models of slowed ageing (Barger, et al., 2015).

A DR transcriptional signature that is shared across multiple tissue types was derived by meta-analysing gene expression profiles from 10 different tissues types (liver, heart, muscle, hypothalamus, hippocampus, white adipose tissue, colon, kidney, lung and cochlea). Such a signature characterizes a common response to DR that includes 28 genes for which expression response to DR is most shared among tissues. This response consists of both activation and inhibition of stress-response pathways (Swindell, 2008d). Co-expression and network properties of DR-regulated genes were investigated using data generated from many microarray profiles. It was found that genes downregulated with DR are highly connected and located in dense network regions, while DR upregulated genes are weakly connected and positioned in sparse network regions (Swindell, 2008c).

The transcriptional responses to dietary restriction in 17 mouse tissue types were examined, as well as the changes with ageing in 22 tissues. DR was found to induce the expression of genes associated with oxidative stress, inflammation and tumourigenesis. Ageing was found to upregulate granulin and secreted phosphoprotein. This lead to the derivation of a common consensus signature of DR and ageing as well as the finding that DR does not simply induce a genome-wide reversal of age-associated gene expression patterns (Swindell, 2009).

A multiple comparison approach using whole genome transcriptional profiles (dietary restriction and genetic and pharmacological DR mimetics) was used to identify genes and pathways involved in DR-mediated lifespan extension. One gene, named *takeout*, that is commonly upregulated under lifespan extending intervention when overexpression is capable of lifespan extension (Antosh, et al., 2011b).

Evolutionary conserved genes were identified in transcriptional profiles of dietary restriction. Subsequent testing of 16 genes that are upregulated under DR led to the discovery of 8 genes that abolish the DR-induced heat stress in *C. elegans* and three were also found to be DR-essential as their mutations abolish the increased lifespan in response to dietary restriction (Barger, et al., 2008a).

Gene expression signatures were derived from gene expression changes that occur across species when lifespan is extended via different interventions. Heterogeneous datasets from different measurement platforms and organisms were used and a non-parametric methodology that can detect statistical significance of overlaps in ranked lists of genes was applied to estimate the genes with a common expression profile (Antosh, et al., 2011a).

A meta-analysis of dietary restriction transcriptional gene expression profiles in mammals was performed. DR was found to differentially express growth hormone signalling, lipid metabolism and immune response as well as retinal metabolism, copper ion detoxification, and circadian rhythm. A putative regulatory role of *Srebf1* and *Ppara* in the DR response was found (Plank, et al., 2012).

It is controversial whether DR reverses ageing gene expression patterns. Some studies suggested this to be the case while others did not substantiate this. Using comprehensive molecular consensus signatures can reveal light on this controversy (Swindell, 2009). However DR certainly does not totally reverse ageing gene expression changes, as animals upon DR still age, but more slowly.

1.12.10 Cross-Species Signatures

Ageing is an apparently complex multifactorial process during which the different scales of biological systems such as molecules, compartments, cells, tissues and organs undergo changes over time, resulting in loss of function, increased morbidity and eventually death (Fontana, et al., 2010). Most of the current knowledge of the molecular mechanisms of ageing has been derived, to a large extent, from studies on lifespan and longevity of simple model organisms (for example yeast, nematodes, and flies). Many of those mechanisms are conserved in mammals and may thus be relevant to human ageing.

Analysing gene expression from different species is a powerful way to identify evolutionary conserved transcriptional programs. For instance, meta-analysis of microarray data from heat stress experiments performed in eight different species identified several well-known evolutionary conserved transcriptional responses (Kristiansson, et al., 2013). Ageing is assumed to be an extremely well conserved process, so by doing cross-species gene expression meta-analysis the common mechanisms of ageing shared between different species could be identified. Gene expression data from all those species that were profiled for age-related changes could be gathered into cross-species common signatures. Several such signatures could be derived, e.g. from phylogenetically closely-related species and even together with more distantly related species. Life stages and ages have to be aligned to each other for this comparative biology approach. In order to enable this kind of cross-species common signatures, homologous relationships of genes need to be utilized or signatures have to be derived on the basis of functional categories. The latter method is not yet feasible practically as gene annotation is quite different in coverage for different species and in many species it is too sparse. It could be overcome if functional categories can be derived from data itself (i.e. from coexpression data), but this requires extensive omics data that have to be available for all those included organisms. Therefore, meta-analysis based on homologous genes appears to be the most broadly applicable way of accomplishing the creation of such signatures.

1.12.11 Cancer Signatures

Some ageing-related and human embryonic stem cell-related molecules are highly expressed in cancer, which may indicate important mechanisms common to ageing, stem cell and cancer. It has been suggested (Wang, 2013) that cancer is a developmental and evolutionary disease that is strongly related to ageing. Initiation, proliferation and metastasis of cancer is associated with deregulation of stem cells (Wang, 2013). Stem cells themselves are deregulated during ageing (Chambers, et al., 2007; Jung & Brack, 2014; Li, et al., 2011).

It might be intriguing to determine whether certain defined classes of genes have a tendency to be differential expressed or regulated with ageing such as cancer genes, i.e. proto-oncogene and

tumour-suppressor genes. Further it would be insightful to check whether interventions known to interfere with ageing also impact on those classes, e.g. dietary restriction and rapamycin treatment as these are known to postpone cancer incidence and severity.

Mutations accumulate with age at an organ- and tissue-specific rate. The spectrum of age-accumulated mutations differs greatly from organ to organ and similar initial mutation spectra of different tissues diverge significantly over the lifetime of individuals (Vijg, 2004). How the mutation rate of a tissue is related to the rate of change in the transcriptome and epigenome is an intriguing question.

1.12.12 Longevity Signatures

The genetic makeup of the organism dictates the species-specific rate of ageing and the maximum lifespan. The genotype is converted to phenotype through gene expression regulation including epigenetic, transcriptional and translation regulation as well as localization and post-translational modifications. A group of gene regulatory factors (including transcriptional factors and epigenetic modifiers) play a critical role in controlling the initial rate of transcription of specific genes directly by interacting with regulatory sequences at gene promoters and enhancers as well as neighbouring regions (Roy, et al., 2002).

Animals that exhibit maybe negligible senescence like the naked-mole rat (although it still ages and dies) and the long-lived sea urchins do not exhibit alterations in their gene expression profiles with age as much as other organisms do (Kim, et al., 2011; Loram & Bodnar, 2012). Molecular signatures of species that are extremely long-lived, or even exhibit negligible senescence, or declared biological immortal can be used to determine what are the differences that between a species that ages and one that does not.

1.12.13 Biomarkers of Biological Age

The study of ageing and the development of interventions to retard it requires the establishment of effective biomarkers of ageing and thus biological age. Biogerontologists have been seeking for robust biomarkers of ageing for decades, though most putative markers were not better than chronological age. By utilizing molecular signatures however, it is possible to establish predictive biological markers of ageing.

The regulation of gene expression is coupled with the regulation of lifespan (Rogina & Helfand, 1995). The transcriptional stability is an important determinant of longevity (Kogan, et al., 2015). The rate of change in gene expression correlates with changes in longevity under various conditions. Therefore, ageing gene expression signatures may be indicators of physiological age (biological age) and provide thus valuable biomarkers (Seroude, et al., 2002). Similarly, the dietary restriction gene expression signatures can provide valuable biomarkers for anti-ageing interventions that mimic the effect of dietary restriction *per se* on ageing.

Gene expression data can be used to identify candidate genes involved in ageing and longevity as potential differentially expressed genes (Seroude, et al., 2002). Those candidates can be used as biomarkers of ageing and anti-ageing interventions.

The length of telomeres have been proposed repeatedly to be excellent robust molecular biomarkers of ageing. However, the telomere/telomerase system is not limiting lifespan in all ageing organisms equally. For instance, the short lifespan of GRZ strain of fish is not caused by reduced telomerase activity nor accelerated ageing by telomere shortening (Hartmann, et al., 2009).

Gene expression profiling suggests that both DR and anti-ageing compounds such as resveratrol may retard some aspects of ageing through modifications in chromatin structure and transcription (Ludewig, et al., 2014). Methionine restriction seems to decrease the degree of methylation of genomic DNA (Sanchez-Roman, et al., 2011). Profiling of those changes is warranted.

Chromatin states and long-range genomic segments impact on ageing via DNA methylation (Sun & Yi, 2015). Epigenome-wide association studies systematically identify epigenetic variants across the genome that associate with complex phenotypes such as ageing. Such studies exhibit challenges related to methodology, design and interpretation because of the dynamic nature of epigenetic variants over time (Tsai, et al., 2012). The most likely candidate for the long-term adaptive changes mediated by dietary

restriction is the epigenome. It has been suggested that larval nutritional stress may lead to the induction of adaptive epigenetic rearrangements which extends the lifespan (Vaiserman, et al., 2013).

Epigenetic marks are reset twice during the lifetime of most multicellular organisms. Once during gametogenesis and once during early development. Cellular reprogramming involves the erasure of the epigenetic modifications that mark the differentiated state. It is unclear whether reprogramming of cells reverses all signs of cellular ageing or whether there is an *ageing signature* remaining in induced pluripotent stem cells (Rohani, et al., 2014).

In order to understand the ageing process, molecular profiles of ageing, DR and long-lived mutants need to be integrated into molecular signatures spanning different organs and species. Age-related gene expressions changes, although exhibiting tissue-specific components and being heterogeneous, share a common signature across various tissues which, at least to some degree, is coordinated in systemic fashion. Gene expression signatures of ageing are characterized by a reduction in expression of genes related to energy metabolism that is common to multiple tissues and species. It is also clear that ageing and DR signatures are modular in nature as they possess defined transcriptional programmes of coexpression networks. The alteration of the redox state and its modulation by DR appears to be the most conserved process common to multiple mammalian tissues as well as shared with various organisms. Follow-up analyses of these signatures to understand their underlying transcriptional regulation can reveal key regulators and potentially new drug targets [Figure 2 Transcriptional Profiling for Drug Discovery].

DNA microarrays provided the first genome-wide signatures of molecular changes with age and gave insights into the mechanisms governing ageing and its modulation. Emerging next-generation sequencing technologies, however, now allow deep molecular profiling of the transcriptome, DNA-protein interactions and the epigenome (de Magalhaes, et al., 2010). These platforms therefore provide a superior understanding of molecular changes with age. Moreover, a more efficient data analysis and integration, for example with genes previously associated with ageing such as those in the *GenAge* database (de Magalhaes, et al., 2009b), will improve the understanding of the regulation of gene expression changes in ageing, DR and other relevant processes (de Magalhaes, 2009).

Overall, molecular signatures based on microarrays have provided mechanistic insights as well as biomarkers of ageing and DR, and could be used to identify ageing retarding interventions without the need of performing full lifespan experiments. Future studies employing ever more powerful experimental and computational techniques promise to contribute even further to unravel the transcriptional changes during ageing and therefore increase the understanding of ageing mechanisms and how to manipulate them to improve human health.

1.13 Objective

The objective of this work is to apply functional genomics and bioinformatics approaches to the study of ageing and its interferences via dietary (i.e. dietary restriction) as well as pharmacological (i.e. anti-ageing drugs) means in order to gain insights about the underlying mechanisms and predict possible interventions that are likely to slow down ageing and/or reverse age-related changes.

More specifically, the aims of this work are to

1. classify, characterize, and predict ageing genes (i.e. gerontogenes and ageing-suppressor genes) as well as genes essential for lifespan extension mediated by dietary restriction,
2. derive and characterize molecular signatures of ageing and dietary restriction in human as well as model organisms,
3. discover small molecules or drugs that affect the ageing process.

2 Ageing Genes

Abstract: Many genes regulate the ageing process in a positive or negative manner. Gerontogenes can speed up ageing by their normal activity, therefore decrease lifespan if hyperactivated and increase it if inactivated, while ageing-suppressor genes slow down ageing normally, hence decrease lifespan by lack of activity and their overactivation increases lifespan. Here a curated knowledge base that includes loss- and gain-of-function experiments that impact on lifespan of organisms including human, mouse, fruit fly, nematode, and yeast was established. It classifies ageing genes that control the ageing process into two classes: 1) gerontogenes that mediate the ageing process, therefore decrease lifespan of the organism. 2) ageing-suppressor genes that suppress the ageing process, hence increase lifespan of the organism. Gerontogenes and ageing-suppressor genes were found to have very different as well as common associations. Regarding the common associations, gerontogenes and ageing-suppressor genes tend to regulate them in different directions. Gerontogenes were found to be involved in regulation of growth and development, promotion of TOR signalling, translation, and inflammation, while ageing-suppressor genes are involved in stress response, TOR signalling inhibition, MAPK signalling and diverse repair processes as well as proteome homeostasis and chromatin modification.

2.1 Background

Life comes with instructions. An organism is encoded in its genome consisting of genes and regulatory elements as well as structural regions and epigenetic decorations. Genes determine the traits as well as the life cycle including lifespan. Inheritance (i.e. genetics) determines the lifespan of humans at least to approximately 25-32% (Herskind, et al., 1996). The greatest effects on ageing rates appear to be caused by genetic variability, as evidenced by the large variation in ageing and lifespan among related species. For instance, in the primate order, one of the shortest-lived members is the mouse lemur, with a maximum lifespan of about 15 years (when in human care). In contrast, it is not unusual for humans to surpass 90 years. Ageing appears to be controlled greatly by genes and specific genes are affected by environmental influences (Wei, et al., 2008). The lifespan extending effect of dietary restriction can be mimicked and cancelled out by mutating certain genes (Wuttke, et al., 2012).

Many studies have proven that ageing has a genetic basis (Curtsinger, 2007). Some mutations of defined genes cause premature ageing (called progeroid syndromes), where kids suffer from rapid ageing and die of age-related diseases (often even before becoming teenager). In contrast, mutations of other single genes extend the lifespan of animals up to tenfold (Ayyadevara, et al., 2008). Manipulation of only one defined gene can make old organisms young again (Unal & Amon, 2011). However the genes that are involved in the ageing process are poorly classified.

2.1.1 Classification of Ageing Genes

The genetics of ageing has advanced from knowing just a single gene involved in ageing to thousands of genes in different model organisms that are involved in ageing. However, a systematic classification of the genes under study in biogerontology is lacking.

One of the most general concepts is that of an *ageing-associated gene*, which is a class of genes that are associated with the ageing process with some evidence that might be even be only correlative. It is a subclass of a gene. The next important class is the one of the *ageing gene* which affects the rate of ageing (obtained from loss-of-function and gain-of-function evidences). This class can be subdivided into *gerontogene* and *ageing-suppressor gene* (akin to the distinguishment of oncogenes and tumour-suppressor genes in cancer research). Thus there are genes which accelerate ageing (gerontogenes) and genes that antagonize ageing (ageing-suppressor genes). Consequently, suppressing gerontogenes and activating ageing-suppressor genes will have the potential to increase the lifespan and perhaps restore youthfulness.

Ageing genes are assessed by testing whether their genetic perturbation impacts on the lifespan of an organism or not. In general it is expected that decreasing the activity of gerontogenes will increase lifespan, while increasing their activity will decrease lifespan. On the other hand, enhancing ageing-suppressor genes are expected to increase the lifespan, while decreasing their activity will result in shorter lifespan. This classification of ageing-associated genes is shown in [Figure 3 Ageing-Associated Gene Classification].

–

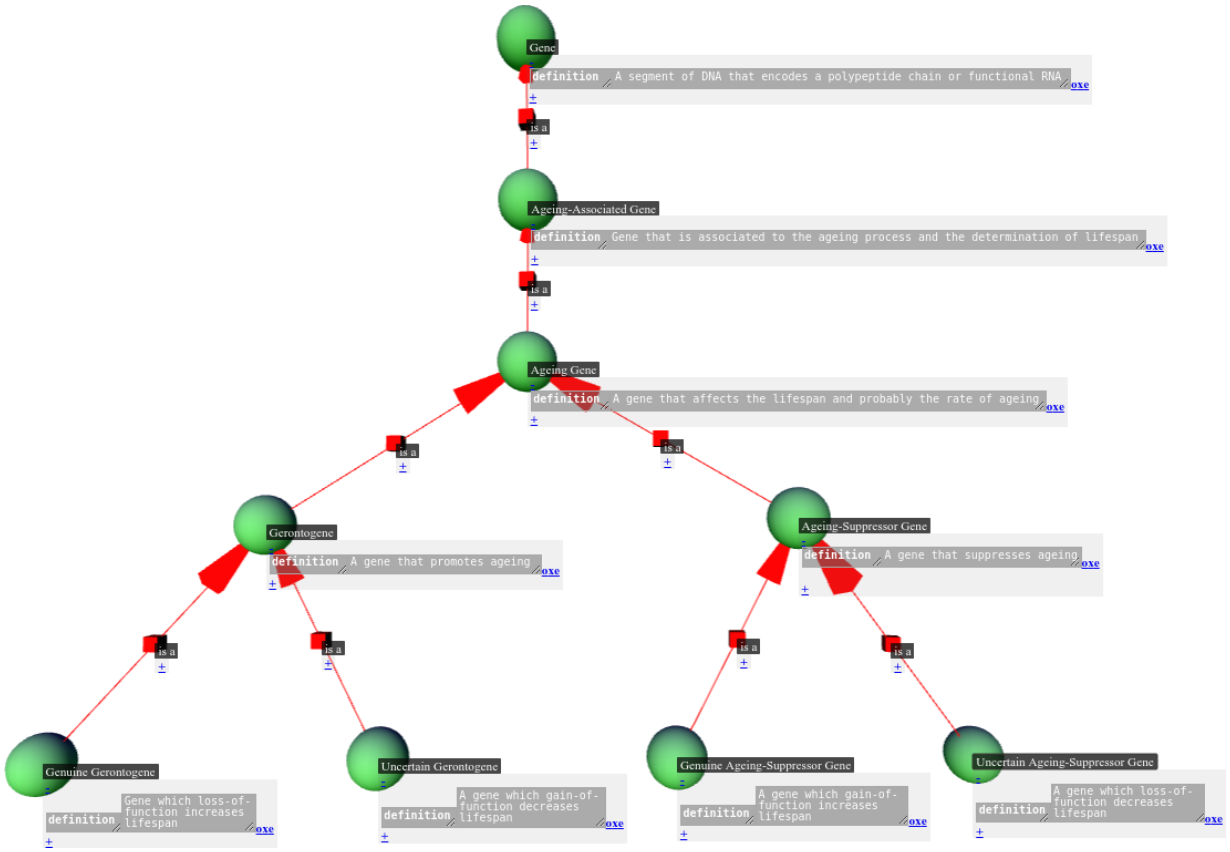

Figure 3: Ageing-Associated Gene Classification*. Classification of ageing-associated genes. This hierarchy provides definitions of ageing gene which is a subclass of ageing-associated gene (which itself is a subclass of a gene). Ageing genes have two discrete subclasses, namely gerontogene which enhances ageing and ageing-suppressor gene which suppresses ageing. Based on the type of evidence gerontogenes and ageing-suppressor genes can be further subclassed into genuine and uncertain ageing genes dependent on whether there is gain- or loss-of-function supporting the assignment and whether there is lifespan extension or shortening associated with those experiments.*

Genuine gerontogenes and genuine ageing-suppressor genes result in lifespan extension by their loss-of-function and gain-of-function, respectively. True positive ageing genes (i.e. genuine ageing genes) therefore are composed of genuine gerontogenes and genuine ageing-suppressor genes. Uncertain ageing genes are (indefinite, indeterminate, potential, or counterfeit; could be false positive) gerontogenes and ageing-suppressor genes which shorten the lifespan upon their overactivation and inactivation, respectively. As increasing lifespan (and especially maximum lifespan) is a much stronger indication of a gene manipulating the ageing process than decreasing lifespan, there is less confidence that uncertain ageing genes are acting on ageing or rather are involved in the limitation of lifespan in some other way (e.g. sickness).

2.1.2 Approaches for Identifying Ageing Genes

2.1.2.1 in vivo

Ageing genes are usually identified via genetic permutation experiments that involve loss- or gain-of-function mutations of a single gene and measuring the lifespan of a cohort of mutant organisms in comparison to a cohort of "wild-type" or otherwise genetic identical control group. A significant change in the lifespan of the mutant organism is an indication for the interfered gene to be an ageing gene.

Common biomedical model organism used for lifespan experiments are *Saccharomyces cerevisiae* (budding yeast), *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly) and *Mus musculus* (house mouse). In yeast ageing genes can be identified via replicative and chronological lifespan assays even in an automated fashion (Jo, et al., 2015). In nematode individuals are declared as dead if they stop moving in an agar plate Petri dish or liquid culture. Those animals are synchronized prior to the lifespan experiment and usually unfertilized via either genetic modification or chemical treatment (with Floxuridine; FUdR). Lifespan assays can be performed in an automatic fashion via modified document scanners (Stroustrup, et al., 2013). For fruit flies larvae are synchronized to provide age-matched adults for analysis. Newly hatched males and females are allowed to mate for two days before they are separated into veils in constant density so gender specific effects can be measured. Throughout their lives the food supply is separated nor more less than every two to three days and death is recorded in order to document the time course of mortality (Linford, et al., 2013). Mice are housed in a special facility called a vivarium and hold in groups of the same age. Food and water is normal provided at *ad libitum* and cages are cleaned regularly. Enrichment is provided within the cages, like running wheels in order to allow physical activity. Death mice are counted and removed from the cages regularly. Lifespan experiments in fruit fly and mouse have yet to be automated.

Rather than lifespan, other phenotypes, like age of maturation, have been suggested as surrogate for identifying ageing genes (Flurkey & Yuan, 2012). However, lifespan remains the gold standard for assaying effects on ageing.

2.1.2.2 in silico

Identifying new ageing genes is basically a problem of class prediction. Data sources like annotations, interactions, and omics data can be used to derive features for predictive algorithms. Already known ageing genes can be used to feed into such an algorithm to predict novel ageing genes.

2.1.2.2.1 Genomics

Genomic data can be used as inputs. A genome-wide linkage study (GWLS) is used for genetic mapping and to identify loci associated with a trait. It measures markers and trait values in families and sees which segregate similarly to identify the underlying loci of the trait.

A genome-wide association study (GWAS), also known as whole genome association study (WGA study, or WGAS), is an examination of many common genetic variants in different individuals to see if any variant is associated with a trait. GWAS typically focus on associations between single-nucleotide polymorphisms (SNPs) and traits like major diseases.

Different omics data such as GWLS, GWAS and genome-wide expression profiling if integrated with interactomics can be used for rank-based gene prioritization. Common genetic signals obtained from different platforms might serve as a robust markers for evaluating the role of established genes and identifying new genes involved in a certain process of interest (Talwar, et al., 2014).

An increasingly amount of genes are found to be associated with longevity as well as with age-related disease. Age-related disease genetic variants help to inform on identifying longevity-associated variants and genes (Fortney, et al., 2015).

2.1.2.2.2 Interactomics

The genes in a genome give rise to biological networks on several levels of abstractions. The interaction between genes (genetic interactions; epistasis) as well as their gene products (physical interactions or complexes) have been used for characterising their associations and for predicting candidate genes including those on ageing. Several attempts to utilize interactomics data have been made so far with fruitful insights.

The interactions of gene products of lower model organisms ageing genes have been found to follow a power law distributions, i.e. there are only few genes with many connections (so called hubs) while the majority have only a few connections. Proteins of ageing genes have a significant higher connectivity than expected by chance (Promislow, 2004). As ageing genes have been confirmed to exhibit this trend of higher number of connections (i.e. they tend to be hubs), it has been suggested to use this network property to prioritize candidates (Ferrarini, et al., 2005). This concept has been adapted to other model organisms and used to make ranked list of candidate ageing genes (Witten & Bonchev, 2007). Genes found to affect the lifespan of model organisms have been repeatedly and independently compiled and human orthologs used to project their functions (under the assumption that ageing is a highly conserved process across phyla (Smith, et al., 2008). Most of the hubs in networks of genes derived from orthologs of model organisms ageing genes have been found to be involved in at least one and many in several age-related diseases (Budovsky, et al., 2007). The first order interactors of known ageing genes are likely to participate in the regulation of ageing more than random chosen genes. Networks in worm and humans have been found to pose high predictive power for identifying ageing genes (Tacutu, et al., 2012). In such first-order networks of ageing genes, hubs and centrally located nodes have higher likelihoods of being associated with ageing and longevity than do randomly selected nodes. Essential genes for living have been used as another criteria to prioritize candidate selection (Tacutu, et al., 2012). Rules have been manually crafted from different sources of evidence to evaluate whether a given gene is likely to be a ageing gene (Callahan, et al., 2015). Besides this, Nearest Neighbourhood, Decision Trees, Naive Bayes and Support Vector Machine Classifier have been trained on predicting ageing genes, and therefore eliminating the need for handcrafted rules (but not feature engineering) (Wan, et al., 2015; Fabris, et al., 2015). Ageing and age-related diseases have again been found to share more genes than expected by chance. Genes that promote ageing have been found to be associated with different age-related diseases than those that suppress ageing (Fernandes, et al., 2016). Protein complexes involved in certain processes can be predicated from networks as well by including interactions between protein complexes (Le, 2015).

In graph theory and network analysis, indicators of centrality identify the most important nodes within a graph. Such indicators include node degree, closeness, betweenness, and eigenvector centrality.

The simplest centrality measure is based on node degree, i.e. the number of edges connected to a node. In connected graphs there is a natural distance metric between all pairs of nodes, defined by the length of their shortest paths. The farness of a node is defined as the sum of its distances from all other nodes, and its closeness is defined as the reciprocal of the farness.

Betweenness centrality quantifies the number of times a node acts as a bridge along the shortest path between two other nodes. Eigenvector centrality (eigencentrality) is a measure of the influence of a node in a network that assigns relative scores to all nodes in the network based on the concept that connections to high-scoring nodes contribute more to the score of the node in question than equal connections to low-scoring nodes.

The guilt-by-association principle states that nodes that have unusual high number of edges with nodes that are members of certain class are also likely to belong to this class. In such, it can be used to find potential additional members of a class like ageing genes.

In addition to node degree, other metrics like closeness, betweenness, and eigenvector centrality can be used for guilt-by-association of interactomes to predict candidate nodes. The higher the centrality to the seed nodes the higher the chance for the node to be related to the seed nodes (Witten & Bonchev, 2007).

2.2 Methods

Genes that were found to impact lifespan upon their genetic perturbation were compiled from the literature for most of the common biomedical model organisms, including *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (worm/nematode), *Drosophila melanogaster* (fruit fly), and *Mus musculus* (mouse) as well as *Homo sapiens* (human). As information in public databases like *Aging Genes and Interventions Database* (Kaeberlein, et al., 2002), *GenAge* (de Magalhaes & Toussaint, 2004), *Lifespan Observations Database* (Olsen & Kaeberlein, 2017), and *AgeFactDB* (Huhne, et al., 2014) among others are distributed to many portals, not up-to-date, inaccurate and not in a proper graph format, references of those resources were looked up and related articles in PubMed/MEDLINE were followed. The compiled list of genes were classified into genuine/uncertain gerontogenes and ageing-suppressor genes.

Guilt-by-association based on physical and genetic interactions (derived from BioGRID Version 3.4.140) was performed to assess genes for their association with ageing. This was done for individual subclasses as well as the superclass ageing genes.

For guilt-by-association a *ratio* was first calculated which corresponds to the number of interactions with the genes in the defined classes divided by the number of all genes it interacts with. It therefore gives a measure of specificity and corrects for the number of connections (i.e. being a hub). The higher the ratio is to one, the more specific does the gene interact with genes within the defined class. In order to test whether the specificity is higher than expected by chance, the hypergeometric distribution is utilized, which is a standard approach (Eden, et al., 2009). Given the total number of genes N in the genome of a species, with B genes interacting with a particular gene and n of these genes belonging to the seed set of interest, then the probability that b or more genes from the seed set are associated with this specific gene is given by the hypergeometric tail:

```
Prob(X>=b) = hypergeometric_test(b, N, B, n)
           = Sum(min(n,B), i=b)
             ((n, i) × (N-n, B-1)) / (N, B)

N = Total number of genes in a genome
B = Total number of interactors
b = Seed genes that are interactors
n = Seed genes
```

The p-values from the guilt-by-association were corrected for multiple hypothesis testing via the Benjamini-Hochberg correction (Benjamini & HochBerg, 1995). These corrected values are termed q-values.

In an ontology, a member (subclass or instance) of a class inherits all relations of its superclasses/types ("true path rule") (Valentini, 2009). For functional enrichment the ontological information about processes, functions and location of gene products is utilized and all implicit relations were made explicit during reasoning. Therefore, in a hierarchy of classes there exists a redundancy for annotations (hierarchical redundancy).

Similar functional enrichment can be tested for in the same way as associating genes. Given again the total number of genes N in the genome, with B genes associated with a particular term and n of these genes in the seed set, then the probability that b or more genes from the seed set are associated with the given term is also given with the same equation. Here as reference set, all genes within this species, annotated with this kind of property are used.

For functional enrichment, ontologies of genes about biological processes (processes), molecular functions (activities), and cellular components (locations) are used which are directed acyclic graphs with edges like "is a", "part of", "regulates", etc. (Ashburner, et al., 2000). The respective annotations were retrieved from NCBI FTP (File Transfer Protocol) server. Orthologs were retrieved from NCBI HomoloGene also via FTP. The *Database for Annotation, Visualization and Integrated Discovery* (DAVID) cope with this kind of hierarchical redundancy by using clustering of semantic similar terms (i.e. redundant and heterogeneous terms are clustered into groups) and condensing large gene lists into gene functional

groups. More specifically it measures relationships among the annotation terms based on the degrees of their co-association genes to group the similar, redundant and heterogeneous annotation contents from the same or different resources into fuzzy annotation groups (Huang, et al., 2007; Huang, et al., 2009a; Huang, et al., 2009b). Here in this work clustering by automatic graph layout, based on force directed graph layout, is utilized. The force-directed graph layout algorithm draws graphs in an aesthetically pleasing way. Its purpose is to position nodes of a graph in a two- or three-dimensional space so that all edges are of more or less equal length and there are as few crossing edges as possible. This is achieved by assigning forces among the set of edges and nodes, based on their relative positions, and then using these forces to simulate the motion of the edges and nodes or to minimize their energy (Kobourov, 2012).

The significant genes and associations are visualized together with the ageing genes as interaction networks by using a force-directed graph layout. Unconnected nodes were connected via shortest path algorithm. Corrected p-values (q-values derived by the Benjamini-Hochberg correction as described above) of the significance to be an ageing gene or being associated with ageing genes are used for the node size. For this q-values were converted to size scale by adding to the minimum size of 5 the log10 of the q-value. Similar, terms that are significantly associated with certain gene classes were assigned the colour of the respective class combination.

2.3 Results

Associations were assessed for ageing genes as well as specific subclasses (gerontogenes and ageing-suppressor genes) within a species and tested for their significance with other genes, processes, functions, and locations according to the Gene Ontology. Guilt-by-association was applied with a q-value threshold of 0.05 (0.0005 for visualization) if not stated differently. Unconnected nodes were connected via the shortest path algorithm. Nodes were colour coded the following way:

Nodes are grey by default, Gerontogenes are filled light red, ageing-suppressor genes are filled light blue and ageing genes are purple. Processes are green, functions are orange, and locations are yellow. Halos indicate respective significant associations with the same colour code. Association with both gerontogenes and ageing genes is indicated with dark red while ageing-suppressor genes and ageing genes is indicated with dark blue. Membership/association with both gerontogene and ageing-suppressor gene is indicated with brown. Physical interactions are pink, genetic interactions are green and other edges like, *is involved in*, *exhibits* and *is located in* are grey. Node size represents significance. The most obvious associations with relevance are summarized for each species grouped by gene class and type of association in text.

2.3.1 Human

In humans there are a few genes established to be ageing genes (such as *WRN*, *BLM*, *LMNA*, etc.), mostly due to fact that mutations in those genes lead to premature ageing diseases (accelerated ageing), like Hutchinson-Gilford progeria syndrome (HGPS). Additionally there are human genes tested by transgenic overexpression in lower model organisms. All those human ageing genes were collectively characterized for their associated genes, processes, activities and locations [Figure 4 H. sapiens Ageing Gene Network].

Human gerontogenes interact physically specifically with *SENP1*, *CDK2*, *CTCF*, *MAST3*, among others. Human gerontogenes participate in positive regulation of cell ageing, neuronal death, negative regulation of mitochondrial functions, negative regulation of telomere maintenance via telomere lengthening, and neurofibrillary tangle assembly.

Human ageing-suppressor genes physically interact specifically with with *H2AFX*, *BLM*, *DKC1*, *ATM*, *WRN*, *TP53*, among others. Human ageing-suppressor genes participate in ageing, stress response, replicative senescence, telomeres/telomerase, DNA repair, regulation of protein stability, negative regulation of apoptotic process, positive regulation of transdifferentiation, and negative regulation of production of siRNA involved in RNA interference. Human ageing-suppressor genes are located primarily in the nucleus, nucleoplasm, nucleolus, nuclear chromosome telomeric region, but also extracellular exosome, RNA-directed RNA polymerase complex, mitochondrion, TERT-RMRP complex, Golgi apparatus, and focal adhesion.

Human ageing genes participate in stress response, mitotic nuclear envelope disassembly, mitotic nuclear envelope reassembly, protein SUMOylation, and regulation of autophagy. Human ageing genes exhibit activities related to protein and DNA binding. Human ageing genes are located in the nucleoplasm, telomere, and mitochondrion.

–

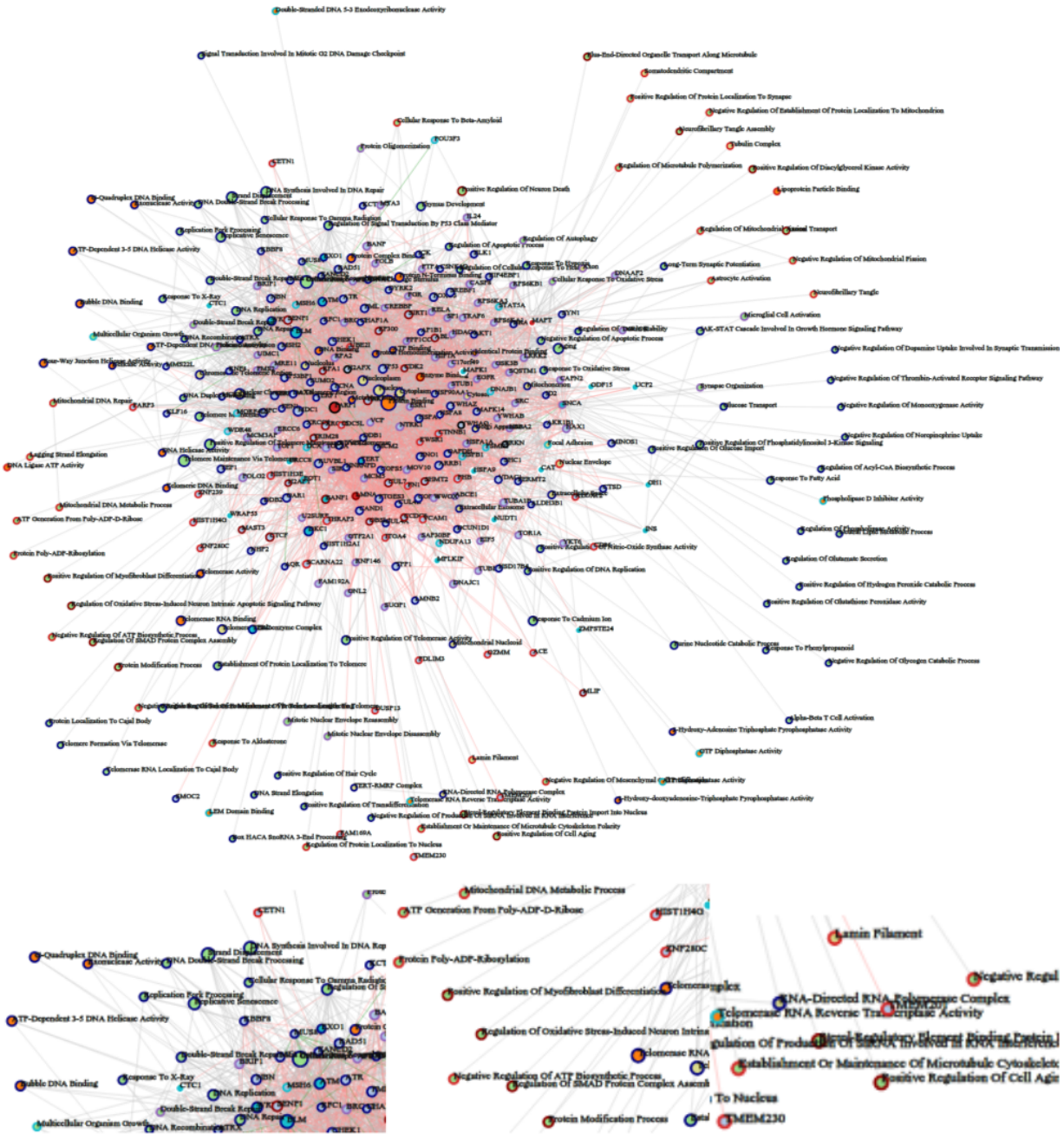

Figure 4: H. sapiens Ageing Gene Network. *Network of human ageing genes.*

2.3.2 Mouse

Mouse, as a mammal, is the closest model organism to human that has been extensively studied for the genetics of ageing. The ability to create transgenic mice via embryonic stem cells and the possibility of conducting lifespan experiments within a few years (3-4 years) has lead to the discovery of quite some genes to be involved in ageing. Novel ageing genes in mouse were predicated via the guilt-by-association concept with murine ageing genes as seeds. Murine ageing genes, gerontogenes and ageing-suppressor genes were characterized for their enrichment in processes, functions, and locations respectively [Figure 5 M. musculus Ageing Gene Network].

–

***Figure 5: M. musculus Ageing Gene Network**. Network of mouse ageing genes.*

Mouse gerontogenes participate in (cell) ageing and determination of adult lifespan, insulin/IGF1 and growth hormone signalling, positive regulation of glucose import, gene regulation, growth and cell cycle, apoptosis, TOR signalling, positive regulation of MAPK cascade, positive regulation of reactive oxygen species metabolic process, regulation of telomere maintenance, inflammatory response, negative regulation of proteostasis. Mouse gerontogenes exhibit insulin-like growth factor receptor binding, various activities related to gene regulation, translation elongation factor activity, ubiquitin protein ligase binding,

and signal transducer activity. Mouse gerontogenes are located in insulin receptor complex, transcription factor complex, replication fork, and nucleolus.

Mouse ageing-suppressor genes participate in ageing, stress response, telomere/telomerase, DNA repair, circadian clock, cellular senescence, free radicals, stem cell control, proteostasis, positive regulation of canonical Wnt signalling pathway, wound healing, gene regulation, negative regulation of ERK1 and ERK2 cascade, epigenetics, and negative regulation of TOR signalling. Murine ageing-suppressor exhibit telomerase activity, helicase, transcriptional activity, proteostatic activity, chromatin remodelling activity. Murine ageing-suppressor genes are located in nucleus, nucleoplasm, nuclear chromosome telomeric region, (nuclear) chromatin, nuclear matrix, transcription factor TFIID complex, perinuclear region of cytoplasm, and chromatoid body as well as extracellular exosome.

Mouse ageing genes are involved in translation, development, growth, MAPK cascade, positive and negative regulation of telomerase. Mouse ageing genes exhibit E-box binding RNA polymerase II core promoter sequence-specific DNA binding, RNA polymerase II core promoter proximal region sequence-specific DNA binding, and transcription corepressor activity. Mouse ageing genes are located in nuclear envelope, nuclear euchromatin, and CHOP-C/EBP complex as well as autophagosome.

2.3.3 Fly

The easy of genetic manipulation of fruit fly allowed researchers to identify a number of ageing genes. Guilt-by-association was applied with fly ageing genes as seeds to predict novel ageing genes in fly. Ageing genes in fruit fly were assessed for their associated processes, functions and locations [Figure 6 D. melanogaster Ageing Gene Network].

Fly gerontogenes participate in processes related to growth and development and signalling as well as apical protein localization, and positive regulation of immune response, negative regulation of autophagy, and histone H3-K27 methylation. Fly gerontogenes exhibit protein and PI3K binding, core promoter binding, and histone methyltransferase activity (H3-K9 specific). Fly gerontogenes are located in ESC/E(Z) complex, nuclear inner membrane, nucleus, histone methyltransferase complex and mitochondria.

Fly ageing-suppressor genes participates in stress response, protein homeostasis, as well as negative regulation of signalling and growth. Fly ageing-suppressor genes exhibit NAD binding as well as antioxidant and (NAD-dependent) histone deacetylase activity. Fly ageing-suppressor genes are located in microtubule associated complex, nucleus, AP-3 adaptor complex, and HOPS complex, TSC1-TSC2 complex, and pre-autophagosomal structure.

Fly ageing genes are involved in gene silencing, regulation of histone acetylation, positive/negative regulation of transcription DNA-templated, and TORC1 signalling, exhibit transcription regulatory region sequence-specific DNA binding, and are located in nucleoplasm, Sin3 complex, and peroxisome.

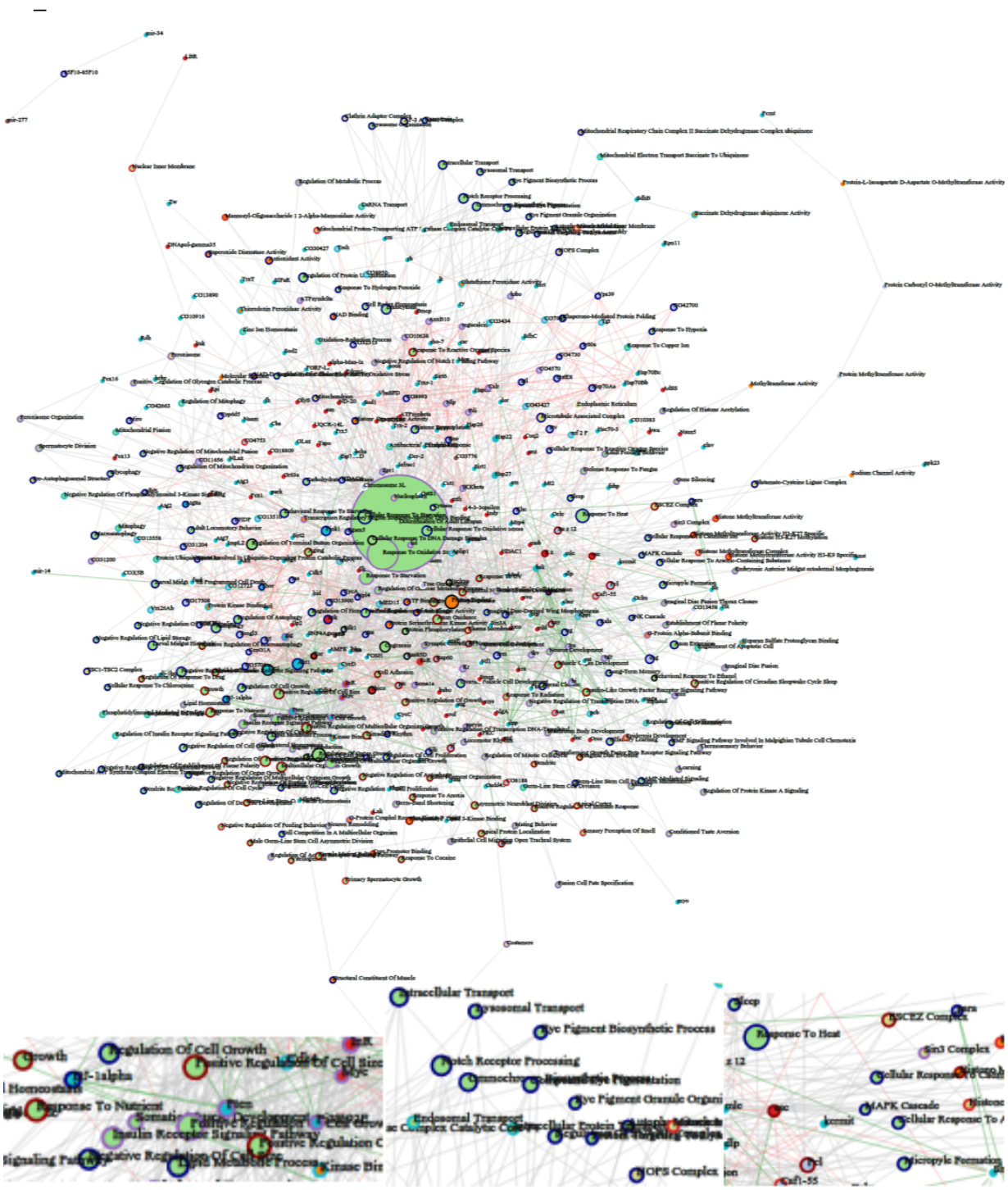

Figure 6: D. melanogaster Ageing Gene Network*. Network of fruit fly ageing genes.*

2.3.4 Nematode

In *Caenorhabditis elegans* genetic interference is extremely easy due to the possibility to simply feed this animal with plasmids expressing RNA interference constructs against defined genes. This has led to the discovery of a plethora of ageing genes. Guilt-by-association was applied on nematode ageing genes to find novel ones. Processes, functions and locations enriched for nematode ageing genes were investigated [Figure 7 C. elegans Ageing Gene Network].

Worm gerontogenes participate in determination of adult lifespan, development, translation as well as growth, TOR, apoptosis, cell division, protein homeostasis, and gene regulation. Worm gerontogenes exhibit translational activity, transcriptional activity, signalling, ATP-dependent RNA helicase activity, and hormone activity. Worm gerontogenes are located in ribosome, TORC2 complex and mitochondrion, and nuclear envelope.

–

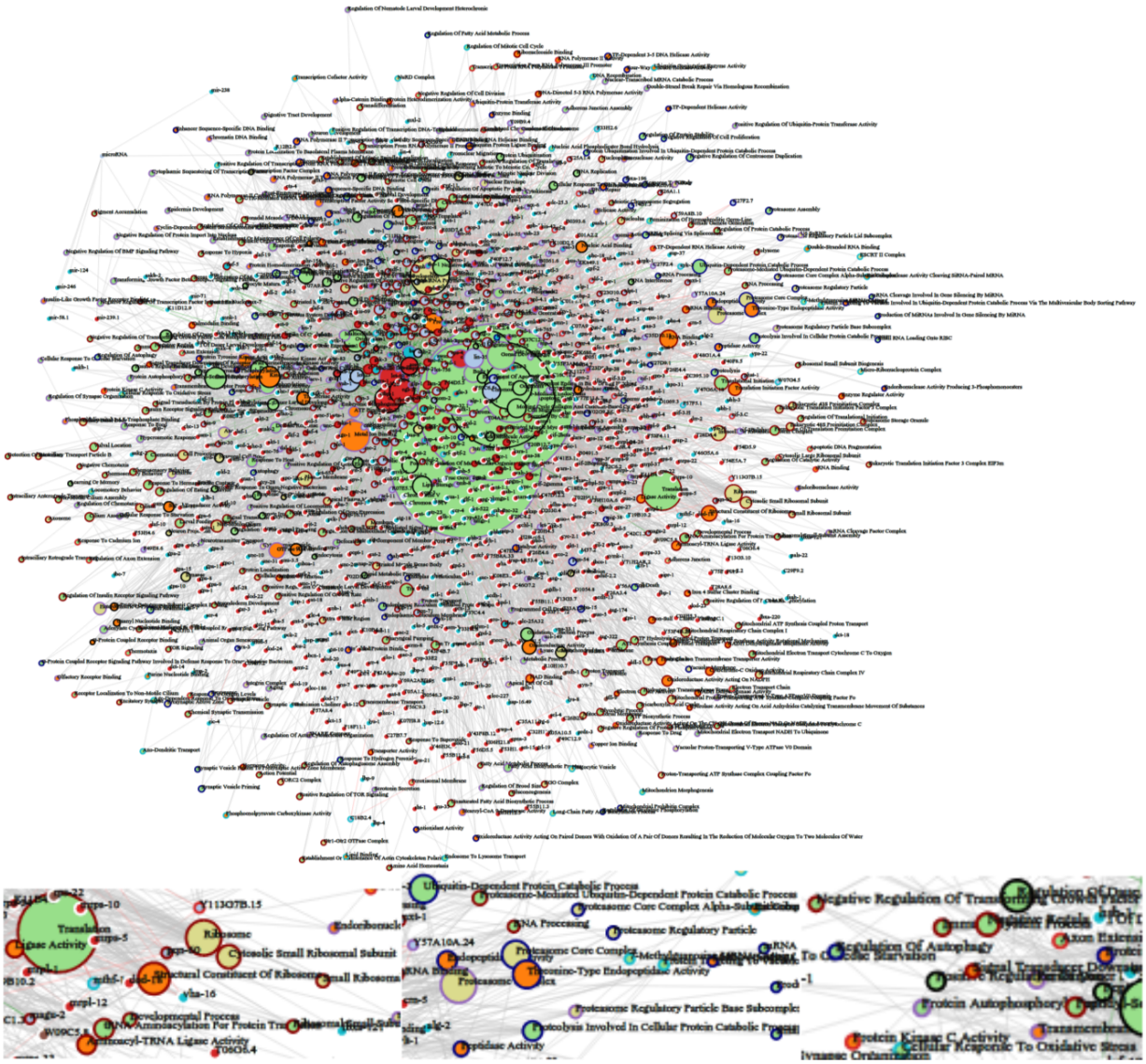

***Figure 7: C. elegans Ageing Gene Network**. Network of nematode ageing genes.*

Worm ageing-suppressor genes participate in adult lifespan determination, transcription regulation, RNA interference, and stress response as well as proteostasis. Worm ageing-suppressor genes exhibit gene regulatory functions, helicase, antioxidant activity, RNAi, peroxiredoxin activity, proteostatic activity. Worm ageing-suppressor genes are located in proteasome, vacuolar membrane, phagocytic vesicle, nuclear envelope, ESCRT II complex, transcription factor complex, and NuRD complex.

Worm ageing genes are involved in ageing, programmed cell death, positive regulation of ubiquitin-protein transferase activity, negative regulation of transcription factor import into nucleus, nuclear-transcribed mRNA catabolic process, regulation of actin cytoskeleton organization, and animal organ senescence, exhibiting DNA repair, chromatin DNA binding, ubiquitin-protein transferase activity, actin binding, DEAD/H-box RNA helicase binding, and are located in vacuolar proton-transporting V-type ATPase V0 domain, lysosome, and proteasome regulatory particle base subcomplex.

2.3.5 Yeast

–

***Figure 8: S. cerevisiae Ageing Gene Network**. Network of yeast ageing genes.*

Saccharomyces cerevisiae, as a unicellular model organism with only about 6500 genes and the availability of deletion and overexpression mutant libraries, enabled researchers to discover so far the largest number of ageing genes in a single organism. Ageing genes in budding yeast were characterized for their enriched associated processes, functions, and locations. Yeast ageing genes were used as seeds for guilt-by-association [Figure 8 S. cerevisiae Ageing Gene Network].

Yeast gerontogenes participate in translation, and isocitrate metabolic process, MAPK cascade involved in cell wall organization or biogenesis, and regulation of cell size. Yeast gerontogenes exhibit ribosomal and DNA binding. Yeast gerontogenes are located primarily in ribosome as well as actin cortical patch.

Yeast ageing-suppressor genes participate in telomere maintenance, epigenetics, positive regulation of RNA polymerase II transcriptional preinitiation complex assembly, DNA repair, autophagy, and proteolysis. Yeast ageing-suppressor genes exhibit ATP-dependent DNA helicase activity, protein kinase activity, protein serine/threonine kinase activity, and proteasome-activating ATPase activity. Yeast ageing-suppressor genes are located in telomeres, and proteasome.

Yeast ageing genes participate in replicative and chronological cell ageing, age-dependent response to oxidative stress involved in chronological cell ageing, maintenance of cell polarity, chromatin silencing at telomere, regulation of endocytosis, mitochondria-nucleus signalling pathway, exhibit oxysterol binding, histone acetyltransferase activity, and are located in endosome (membrane) and Atg1/ULK1 kinase complex.

2.3.6 Cross-Species

2.3.6.1 Human Orthologs

The human orthologs of model organism were used as seeds for guilt-by-association with a strict q-value cutoff of 5e-20 [Figure 9 H. sapiens Ageing Gene Orthologs Network].

Human gerontogene orthologs participate in translation, respiration, apopotosis, regulation of mRNA stability, and regulation of signal transduction by p53 class mediator. Human gerontogene orthologs exhibit polyA RNA binding, ribosomal translational activity, and NADH dehydrogenase ubiquinone activity, translation initiation factor activity, ubiquitin protein ligase binding, and rRNA binding. Human gerontogene orthologs make up ribosome and are located in nucleus, nucleoplasm, and mitochondrion.

Human ageing-suppressor gene orthologs participate in ageing, stress response, ubiquitin-proteasome system, autophagy, mitochondrion organization, rhythmic process, MAPK cascade, regulation of mRNA stability, negative regulation of transcription DNA-templated, DNA repair, negative regulation of apoptotic process, and Wnt signalling. Human ageing-suppressor gene orthologs exhibit NAD, (double-stranded) DNA, transcription factor, histone deacetylase, and chromatin binding. Human ageing-suppressor gene orthologs are located in nucleoplasm, extracellular exosome, and mitochondrion, proteasome, perinuclear region of cytoplasm, nuclear chromosome telomeric region, and inclusion body.

Human ageing genes orthologs are involved in protein stabilization, regulation of transcription DNA-templated, transcription from RNA polymerase II promoter, response to estradiol, and protein stabilization, exhibit structural constituent of ribosome and translation initiation factor activity, and are located in nuclear chromatin and lysosomal membrane.

2.3.6.2 Common Associations with Ageing Genes Across Species

Significant associations of guilt-by-association applied to ageing genes in individual organisms that are common to multiple organisms are graphed together [Figure 10 Common Ageing Gene Network].

Gerontogenes and ageing-suppressor genes are both commonly across species involved in determination of adult lifespan, multicellular organismal process, single-multicellular organism process, anatomical structure development, single-organism process, developmental process, single-organism developmental process, animal organ development, regulation of developmental growth, and response to extracellular stimulus. Gerontogenes are associated with ageing, growth regulation, translation, apopotosis, signal transduction including TOR, and transcription regulation.

Ageing-suppressor genes are associated with cell ageing, stress response, oxidation-reduction process, regulation of protein stability, lysosome/autophagy (in particular mitophagy), MAPK cascade, negative regulation of insulin receptor signalling pathway as well as antioxidant and helicase activity, ubiquitination, transcription DNA-templated, nucleus, chromatin binding, nuclear matrix, NAD-dependent histone deacetylase activity, and telomeres.

–

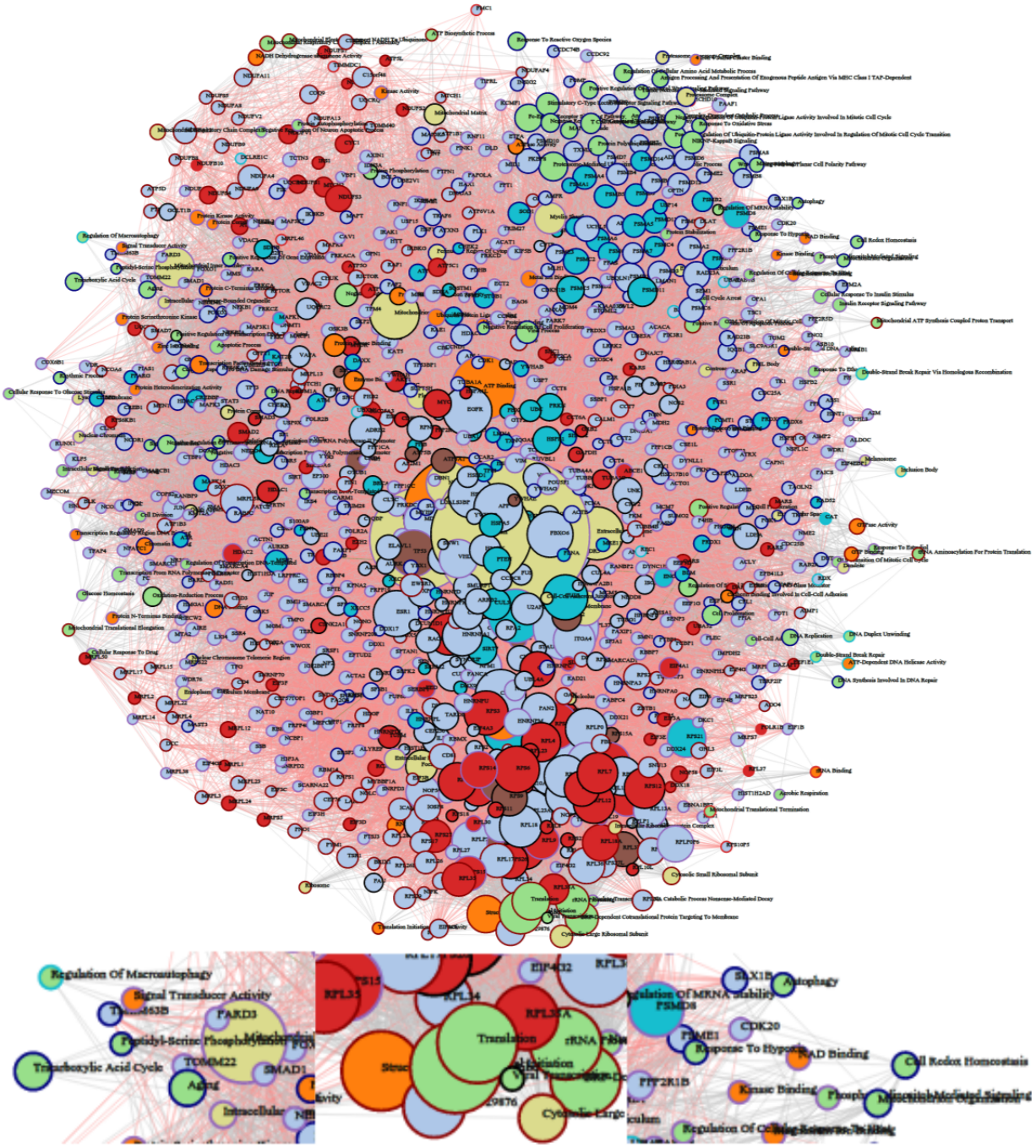

Figure 9: H. sapiens Ageing Gene Orthologs Network. *Network of human ageing genes orthologs.*

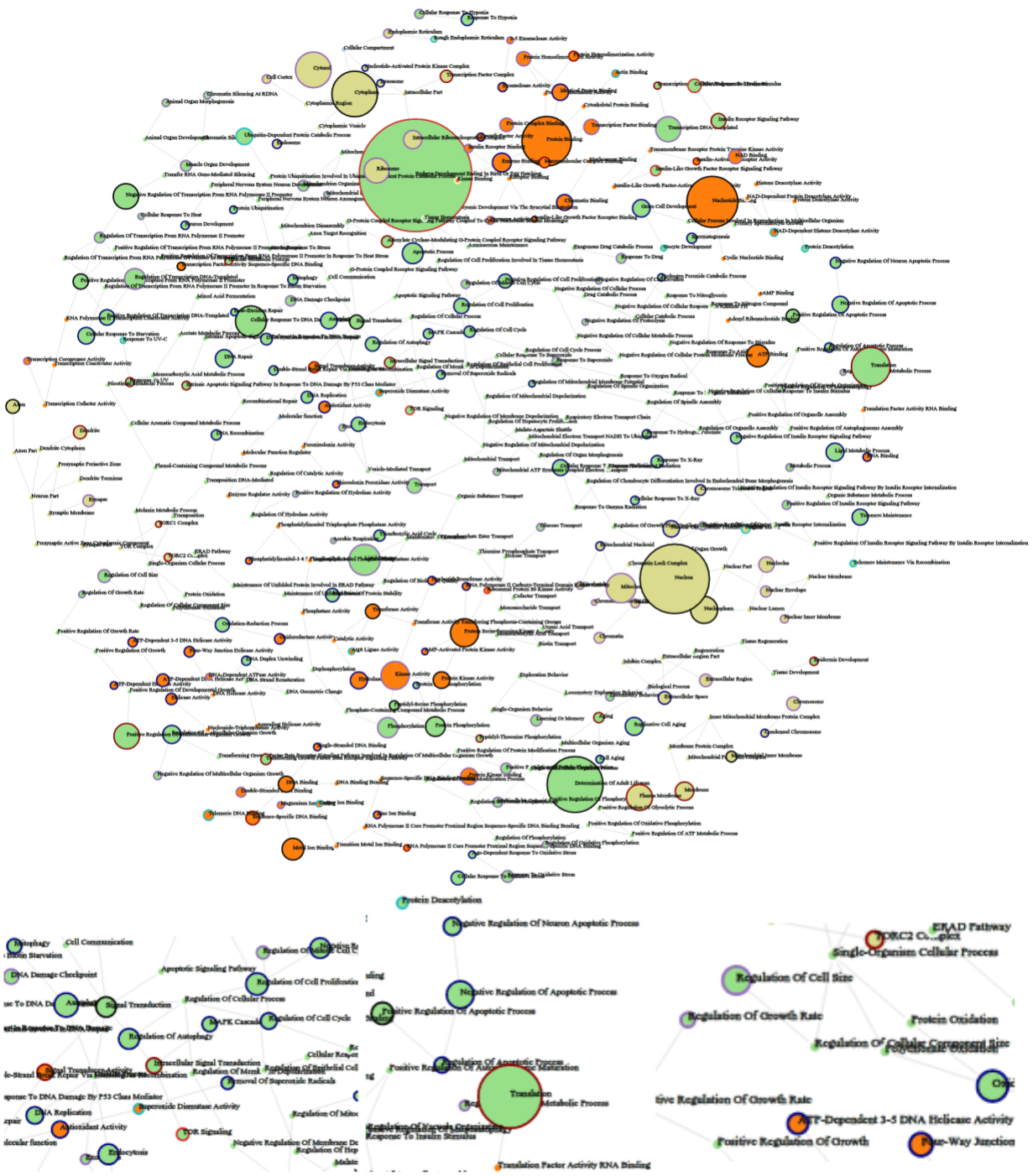

***Figure 10: Common Ageing Gene Network**. Network of associations with ageing genes commonly shared between multiple species.*

2.4 Discussion

Here the class of ageing gene was defined as well as discrete subclasses, namely gerontogene and ageing-suppressor gene. Genuine ageing genes are those that if manipulated can lead to lifespan extension (especially maximum lifespan extension). Ageing genes in commonly used biomedical model organisms and humans were characterized. By doing so, processes, activities and locations (based on the Gene Ontology) related to already known ageing genes and more specifically only for either of its subclasses (gerontogenes and ageing-suppressor genes) were identified. By utilizing interactomics data, a guilt-by-association method was used to find novel ageing genes. Genes not yet known to be involved in ageing but ranked high (low q-value; big node size) are highly likely to be involved in ageing as well. Semantics and causality are still missing.

For each of the commonly used biomedical model organism as well as human guilt-by-association was applied with the known ageing genes as seed classified into their respective subclasses. Association that are common to all ageing genes in this different species have been addressed with two approaches. First with retrieving all human homologs of model organism ageing genes and applying guilt-by-association. Secondly by applying first guilt-by-association for each species and than identifying the shared terms.

Numerous of the above results are in accordance with prior literature while others are new. Many associations make sense in light of previous knowledge about ageing while some are surprising and appear novel.

2.4.1 Genome

In mammals, ageing genes are associated with being involved in DNA metabolism and also function in activities related to DNA binding and modification. This is particular true for ageing-suppressor genes [Figure 4 H. sapiens Ageing Gene Network; Figure 9 H. sapiens Ageing Gene Orthologs Network]. They are involved in the maintenance of telomeres [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Gerontogenes however are commonly significantly associated with negative regulation of telomere maintenance, while ageing-suppressor genes are commonly significantly associated with positive regulation of telomere maintenance and telomerase activity [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Telomeres/telomerase is a known ageing system operating in many species to limit cell division and modulate gene expression among other roles (Geserick & Blasco, 2006). Ageing genes, especially ageing-suppressor genes participate also in the response to DNA damage as well as its repair [Figure 4 H. sapiens Ageing Gene Network; Figure 9 H. sapiens Ageing Gene Orthologs Network]. DNA damage is often claimed to be a primary driver of ageing because of its irreversibility. While there are repair systems, certain damages if not repaired timely (like DNA double-strand breaks) lead to mutations which can impact on gene expression, therefore increase transcriptional noise, and may give raise to cancer (Gorbunova & Seluanov, 2016).

Ageing-suppressor genes are commonly significantly involved in nucleotide-excision repair. Ageing-suppressor genes are commonly significantly associated with being involved in DNA repair, base-excision repair, recombinational repair, double-strand break repair, and double-strand break repair via homologous recombination [Common Associations with Ageing Genes Across Species].

Gerontogenes and ageing-suppressor genes are both commonly significantly associated with being located in nuclear chromosome telomeric region. Ageing-suppressor genes are commonly significantly associated with (nuclear and condensed) chromosome, chromosome segregation and organization, and chromosome organization involved in meiotic cell cycle [Common Associations with Ageing Genes Across Species].

2.4.2 Epigenetics

Both gerontogenes and ageing-suppressor genes have significant associations with epigenetics and gene silencing. However, ageing-suppressor genes especially are associated with various aspects of epigenetics, including chromatin [Figure 5 M. musculus Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network].

Gerontogenes are commonly significantly associated with being involved in epigenetic alteration [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network] and regulation of gene expression epigenetic [Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Similar but to a greater extent, ageing-suppressor genes are commonly significantly associated with epigenetic alteration [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10: Common Ageing Gene Network], epigenetic modification [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network], and regulation of gene expression epigenetic [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network], as well as negative regulation of gene expression epigenetic [Figure 5 M. musculus Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network].

The plasticity of ageing suggest that lifespan may be controlled epigenetically by specific alterations in chromatin state (Greer, et al., 2010). During ageing, changes in the chromatin state alter gene transcription and result in expression of genes that are normally silenced (Jiang, et al., 2013).

2.4.2.1 Chromatin

Gerontogenes are commonly significantly associated with chromatin, nuclear chromatin as well as chromatin binding, chromatin organization, covalent chromatin modification, and regulation of chromatin organization [Figure 10 Common Ageing Gene Network].

Ageing-suppressor genes are commonly significantly associated with chromatin, chromatin binding, chromatin assembly, chromatin assembly or disassembly, chromatin silencing at rDNA, chromatin organization, regulation of chromatin silencing, regulation of chromatin silencing at telomere, covalent chromatin modification, heterochromatin organization, nuclear chromatin, nuclear heterochromatin, regulation of chromatin organization, nuclear telomeric heterochromatin, chromatin remodelling, positive regulation of chromatin organization, heterochromatin, chromatin silencing, negative regulation of chromatin organization, telomeric heterochromatin, and chromatin silencing complex [Figure 10 Common Ageing Gene Network] as well as chromatin organization involved in regulation of transcription [Figure 8 S. cerevisiae Ageing Gene Network].

The repressive chromatin environment at telomeres gives rise to the telomere position effect, which is the epigenetic silencing of telomere-proximal genes (Tennen, et al., 2011). Silencing of ribosomal DNA (rDNA) is tightly linked to suppression of rDNA recombination and the control of cellular lifespan (Machin, et al., 2004).

2.4.2.2 DNA Methylation

Ageing genes including gerontogenes and ageing-suppressor genes are significantly associated with DNA methylation [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network] as well as regulation of DNA methylation [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network].

Ageing-suppressor genes are commonly associated with DNA methylation [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network], DNA methylation or demethylation [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; [Figure 10 Common Ageing Gene Network], and regulation of DNA

methylation [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 10 Common Ageing Gene Network].

DNA methylation involved in embryo development is associated with ageing-suppressor genes [Figure 6 D. melanogaster Ageing Gene Network] as well as maintenance of DNA methylation [Figure 5 M. musculus Ageing Gene Network], while gerontogenes significantly associate with regulation of DNA methylation [Figure 4 H. sapiens Ageing Gene Network]. Ageing-suppressor genes are associated with DNA hypermethylation [Figure 6 D. melanogaster Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 10 Common Ageing Gene Network] and in particular hypermethylation of CpG island [Figure 5 M. musculus Ageing Gene Network]. Overall, ageing-suppressor genes are associated with methylation-dependent chromatin silencing [Figure 5 M. musculus Ageing Gene Network].

Gene silencing in general was shown to be correlated with lifespan extension. Loss of gene silencing is a component of normal ageing observed in multiple organisms (Jiang, et al., 2013).

2.4.2.3 Histones

Gerontogenes are commonly significantly associated with histone modification [Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Ageing-suppressor genes are commonly significantly associated with histone binding and modification, histone mRNA catabolic process, regulation of histone modification, and negative regulation of histone modification [Figure 10 Common Ageing Gene Network].

H2AFX is the most significant candidate of a human ageing gene and ageing-suppressor gene (but not gerontogene) based on physical interactions. H2AFX was found to be associated with longevity in humans (Soerensen, et al., 2012). The histone protein family member X (H2AFX) is important in maintaining chromatin structure and genetic stability (Lu, et al., 2008). H2AFX knockout mice are growth retarded and exhibit signs of genomic instability, a hallmark of ageing (Celeste, et al., 2002). Therefore H2AFX might be an ageing-suppressor gene.

2.4.2.4 Histone Acetylation

Both gerontogenes and ageing-suppressor genes are involved in regulation of histone acetylation [Figure 6 D. melanogaster Ageing Gene Network]. Gerontogenes are associated in positive regulation of histone acetylation [Figure 6 D. melanogaster Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network] while ageing-suppressor genes are associated in negative regulation of histone acetylation [Figure 4 H. sapiens Ageing Gene Network]. Ageing-suppressor genes are significantly associated with negative regulation of histone H4-K16 acetylation [Figure 5 M. musculus Ageing Gene Network].

Ageing-suppressor genes are commonly significantly associated with histone acetylation, histone acetyltransferase activity, regulation of histone acetylation, including negative regulation of histone acetylation [Figure 10 Common Ageing Gene Network]. Specifically, ageing-suppressor genes are commonly significantly associated with being involved in histone H3 acetylation, regulation of histone H3-K9 acetylation, regulation of histone H3-K14 acetylation, and negative regulation of histone H3-K14 acetylation [Figure 10 Common Ageing Gene Network].

2.4.2.5 Histone Deacetylation

Ageing-suppressor genes are significantly associated with histone deacetylation [Figure 8 S. cerevisiae Ageing Gene Network], including histone deacetylase binding [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network], protein deacetylase activity, histone H3 deacetylation [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network], NAD-dependent histone deacetylase activity [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 10 Common Ageing Gene Network] as well as NAD-dependent histone deacetylase activity H4-K16 specific [Figure 8 S. cerevisiae Ageing Gene Network].

Ageing-suppressor genes are commonly significantly associated with histone deacetylation, histone deacetylase complex, histone deacetylase activity, NAD-dependent histone deacetylase activity [Figure 10 Common Ageing Gene Network].

2.4.2.6 Histone Methylation

Gerontogenes commonly significantly exhibit histone methyltransferase activity, histone-lysine N-methyltransferase activity, and H3-K9 specific histone methyltransferase activity [Common Associations with Ageing Genes Across Species]. Gerontogenes are associated with being located in histone methyltransferase complex [Figure 6 D. melanogaster Ageing Gene Network]. Histone H3-K27 methylation and histone methyltransferase activity H3-K27 specific is significantly associated with gerontogenes [Figure 6 D. melanogaster Ageing Gene Network].

There is a maturation-dependent switch in the expression of functionally homologous proteins of epigenetic modifiers. For instance the ratio of histone methyltransferases, EZH1 to EZH2 is high in aged and low in young. The same is also observed for the polycomb repressive complex 1 subunits CBX7 and CBX8 (Weng, et al., 2014). Ezh2 is associated with ageing-suppressor genes [Figure 5 M. musculus Ageing Gene Network].

Ageing-suppressor genes are commonly associated with methylated histone binding [Figure 10 Common Ageing Gene Network]. Mitochondrial stress causes widespread changes in chromatin structure through histone H3K9 dimethylation which is associated with gene silencing (Tian, et al., 2016).

2.4.2.7 Histone Demethylation

Ageing genes are significantly involved in histone demethylation. Histone H3-K4 demethylation is associated with gerontogenes, trimethyl-H3-K4-specific histone H3-K4 demethylation is associated with ageing-suppressor genes and gerontogenes [Figure 7 C. elegans Ageing Gene Network].

Excess H3K4 trimethylation, a mark associated with active chromatin, is detrimental for longevity (Greer, et al., 2010). An increase in ubiquitylation at H2BK123 and methylation at both H3K4 and H3K79 is observed at telomere-proximal regions of replicative aged yeast cells coincident with decreased Sir2 abundance (Rhie, et al., 2013).

H3K4me2 increases at promoters and enhancers globally during postnatal development and ageing and those correspond to gene expression changes in *cis* that are enriched for stress responses, such as the DNA damage response. In support for this there is an increased expression of SETD and DPY30 in aged rhesus monkey brains (Han, et al., 2012). DPY30 is associated with ageing-suppressor genes [Figure 4 H. sapiens Ageing Gene Network].

2.4.2.8 Histone Phosphorylation

Ageing-suppressor genes are commonly significantly associated with being involved in positive regulation of histone phosphorylation [Figure 10 Common Ageing Gene Network]. This would mean that histone phosphorylation suppresses ageing and histone dephosphorylation could enhance ageing.

Histone phosphorylation decreases with ageing in human lymphocytes (Lutz, et al., 1977). H1 histones, for instance, are normally greatly phosphorylated and the phosphorylation of H1 is implicated in regulation of chromatin remodelling. However, there is a significant age-related dephosphorylation of H1.4 and H1.5 and an increase in heterochromatin protein HP1alpha as a function of donor age in human peripheral lymphocytes. Dephosphorylation of H1 histones may be related to an increase in senescence-associated heterochromatin formation during the *in vivo* ageing of human peripheral blood lymphocytes (Happel, et al., 2008). Ageing-suppressor genes may counteract this trend and the loss of ageing-suppressor activity may be responsible for this type of age-related change.

2.4.2.9 Chromatin Remodellers

Ageing genes, gerontogenes and ageing-suppressor genes are located in the Sin3 complex [Figure 6 D. melanogaster Ageing Gene Network]. Ageing-suppressor genes are located within the NuRD complex [Figure 7 C. elegans Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network] Ageing-suppressor and gerontogenes are located in the ESC/E(Z) complex Figure 6 D. melanogaster Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network].

SIN3A/Sin3a/SIN3 itself is associated with ageing-suppressor genes. Actually in fruit fly it is a known genuine ageing suppressor [Figure 5 M. musculus Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network].

2.4.3 Transcription Regulation

Ageing genes, both gerontogenes as well as ageing-suppressor genes are associated with transcription regulation. These associations are very prevalent and it seems like that gerontogenes and ageing-suppressor genes are both associated with positive and negative regulation of transcription, like they are both modifiers of transcriptional regulation. Overall, ageing-suppressor genes appear to have more associations with transcription regulation than gerontogenes. There are some aspects of transcription regulation that are unique to each class [Figure 10 Common Ageing Gene Network].

Gerontogenes specifically are significantly involved in transcription from RNA polymerase I promoter as well as RNA polymerase II transcription factor activity sequence-specific DNA binding [Figure 10 Common Ageing Gene Network]. RNA polymerase I transcripts ribosomal genes which makes sense given the other associations of gerontogenes found here in promoting translation [Translation].

Ageing-suppressor genes are significantly associated with being involved in stress response transcription regulation (regulation of DNA-templated transcription in response to stress, regulation of transcription from RNA polymerase II promoter in response to stress, and positive regulation of transcription from RNA polymerase II promoter in response to stress) as well as transcription factor binding/activity (transcription factor activity, transcription factor binding, RNA polymerase II transcription factor binding, transcription factor binding, transcription factor activity protein binding, nucleic acid binding transcription factor activity, regulation of sequence-specific DNA binding transcription factor activity, positive regulation of sequence-specific DNA binding transcription factor activity) [Figure 10 Common Ageing Gene Network]. Ageing is associated with reduced capacity to cope with various forms of physiological stress. Old animals do not upregulate stress response when confronted with challenges (Zhang, et al., 2002). Different types of moderate stress can extend lifespan presumably by upregulating repair/maintenance systems (i.e. hormesis) (Anderson, et al., 2016).

2.4.4 Stress Response

Ageing-suppressor genes are strongly associated with stress response [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Stress resistance is known to be strongly correlated with long lifespan as it was confirmed in various phenotypical assays. The resistance to multiple types of stress peaks during early adulthood and declines with advanced age (Dues, et al., 2016). Gain-of-functions of ageing-suppressors might make cells more stress resistant by upregulation of their associated stress responses. This is also the proposed mechanism of how hormesis is capable of lifespan extension (Gems & Partridge, 2008).

2.4.5 Signal Transduction

Ageing genes have significant associations with various kinds of signal transduction and signalling. Gerontogenes associate significantly with signalling so do ageing-suppressor genes. However they do regulate individual signalling pathways in different directions.

2.4.5.1 TOR

Gerontogenes are significantly associated with TOR (TOR signalling [Figure 5 M. musculus Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network], TOR complex [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network], TORC1 complex [Figure 5 M. musculus Ageing Gene Network; Figure 6: D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 10 Common Ageing Gene Network], and TORC2 complex [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network] as well as regulation of TOR signalling [Figure 7 C. elegans Ageing Gene Network] and positive regulation of TOR signalling [Figure 7 C. elegans Ageing Gene Network].

Ageing-suppressor genes are also associated with TOR complex [Figure 5 M. musculus Ageing Gene Network], TORC2 complex [Figure 5 M. musculus Ageing Gene Network], TOR signalling [Figure 6 D. melanogaster Ageing Gene Network], TORC1 signalling [Figure 6 D. melanogaster Ageing Gene Network], regulation of TOR signalling [Figure 5 M. musculus Ageing Gene Network, Figure 6 D. melanogaster Ageing Gene Network; Figure 10 Common Ageing Gene Network], negative regulation of TOR signalling [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 10 Common Ageing Gene Network], negative regulation of TORC1 signalling [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 10 Common Ageing Gene Network].

2.4.5.2 MAPK

Gerontogenes and ageing-suppressor genes are associated with MAPK signalling [Figure 10 Common Ageing Gene Network]. However, ageing-suppressor genes are further associated with regulation including both positive and negative regulation of MAPK cascade [Figure 10 Common Ageing Gene Network]. This may imply that MAPK components are both gerontogenes and ageing-suppressor genes whereas ageing-suppressors modulate components activity. Indeed ERK-MAPK has been found to shown to promote longevity by regulating the localization of transcription factors encoded by ageing-suppressor genes (Okuyama, et al., 2010).

Human ageing gene orthologs most significantly physically interact with NTRK1 encoding neurotrophic tyrosine kinase receptor which is a membrane-bound receptor that, upon neurotrophin binding, autophosphorylates itself as well as members of the MAPK pathway.

2.4.5.3 Wnt & Notch

Wnt and Notch are predominant signalling in stem cells. Ageing-suppressor genes are significantly associated with negative regulation of Notch signalling pathway [Figure 6 D. melanogaster Ageing Gene Network] while gerontogenes are also associated with Notch signalling pathway [Figure 5 M. musculus Ageing Gene Network] and in particular with positive regulation of Notch signalling pathway [Figure 7 C. elegans Ageing Gene Network]. Ageing genes are significantly associated with Wnt signalling [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 9 H. sapiens Ageing Gene Orthologs Network]. Ageing-suppressor genes are associated with positive regulation of Wnt signalling pathway [Figure 9 H. sapiens Ageing Gene Orthologs Network]. It should be noted, that in HGPS there is an upregulation of Notch and downregulation of Wnt (Meshorer & Gruenbaum, 2008). Wnt signalling mediates the regenerator phenotype of the biological immortal organisms like hydra (Heber-Katz, et al., 2006) (Lengfeld, et al., 2009).

2.4.5.4 TGF-Beta

Transforming growth factor (TGF) beta receptor signalling pathway is commonly significantly associated with gerontogenes [Figure 10 Common Ageing Gene Network]. TGF-beta is downregulated under DR (Kim, et al., 2016).

2.4.6 Protein Homeostasis

2.4.6.1 Translation

Gerontogenes significantly associate strongly with translation and ribosomal components [Figure 5 M. musculus Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; Figure 9 H. sapiens Ageing Gene Orthologs Network; Figure 10 Common Ageing Gene Network]. Gerontogenes are significantly associated with translation, cytoplasmic translation, regulation of translation, positive regulation of translation, translation elongation factor activity, regulation of translational elongation, translation factor activity RNA binding, and eukaryotic translation initiation factor 4F complex. Ageing-suppressor genes are associated with regulation of translation and specifically negative regulation of translation and translational initiation [Figure 10: Common Ageing Gene Network]. Ageing-suppressor genes are also significantly involved in circadian regulation of translation [Figure 5 M. musculus Ageing Gene Network].

Inhibiting protein synthesis slows down growth and development but extends lifespan. Dietary restriction also inhibits protein synthesis (Kapahi, 2010).

Ageing-suppressor genes are involved in other processes related to protein homeostasis such chaperones, autophagy and the ubiquitin-proteasome system [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network Figure 9 H. sapiens Ageing Gene Orthologs Network; Figure 10 Common Ageing Gene Network]. Ageing-suppressor genes are strongly associated with positive regulation of autophagy and proteasome, while gerontogenes were found to be associated with negative regulation of those processes [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network].

2.4.6.2 Aggregation

Gerontogenes are significantly associated with being involved in neurofibrillary tangle assembly, which is the aggregation, arrangement and bonding together of a set of components to form a neurofibrillary tangle. Neurofibrillary tangles are basically aggregates of hyperphosphorylated tau protein that are most commonly known as a primary marker of Alzheimer's disease [Figure 4 H. sapiens Ageing Gene Network]. However, their presence is also found in numerous other diseases which are collectively known as tauopathies.

Gerontogenes are significantly associated with aggresome assembly, while ageing-suppressor genes are significantly associated with aggrephagy, a form of macroautophagy that selectively degrades protein aggregates [Figure 5 M. musculus Ageing Gene Network]. Therefore it seems like that gerontogenes are involved in the generation of aggresomes and ageing-suppressor genes are involved in there elimination (Hyttinen, et al., 2014).

2.4.6.3 Lysosome

Although gerontogenes are at least associated to be located in the lysosome [Figure 7 C. elegans Ageing Gene Network], ageing-suppressor genes are associated with lysosome [Figure 4: H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network] lysosomal transport [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network] endosome to lysosome transport, lysosomal transport [Figure 6 D. melanogaster Ageing Gene Network; Figure 7: C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network], protein targeting to lysosome [Figure 6 D. melanogaster Ageing Gene Network], lysosome organization [Figure 6 D. melanogaster Ageing Gene Network], protein localization to lysosome [Figure 6 D. melanogaster Ageing Gene Network], lysosomal lumen acidification [Figure 7 C. elegans Ageing Gene Network], regulation of lysosomal lumen pH [Figure 7 C. elegans Ageing Gene Network], autolysosome [Figure 5 M. musculus Ageing Gene Network], and secondary lysosome [Figure 5 M. musculus Ageing Gene Network] as well as lysosomal microautophagy [Figure 8 S. cerevisiae Ageing Gene Network]. An early increase in lysosomal (vacuolar) pH limits mitochondrial function and lifespan (Hughes & Gottschling, 2012).

Lysosomes are organelles that degrade macromolecules and recycle metabolites as well as participate in diverse processes that regulate cellular homeostasis and lifespan. Longevity requires maintenance of lysosome integrity (Li, et al., 2016).

2.4.6.4 Autophagy

Both ageing-suppressor genes and gerontogenes have significant associations with autophagy, but while gerontogenes are associated with negative regulation of autophagy and macroautophagy, ageing-suppressor genes are associated with autophagy, macroautophagy, nucleophagy, mitophagy, regulation of autophagy, macroautophagy, and mitophagy, as well as positive regulation of autophagy and macroautophagy [Figure 10 Common Ageing Gene Network]. Autophagy is a self-renewal process in cells by recycling redundant materials through lysosomal machinery (Wu & Yan, 2011). Therefore, autophagy is a major clearance mechanism that degrades organelles and large protein aggregates to maintain cell survival and protein homeostasis (Emanuele, et al., 2014).

Autophagy was suggested to be the pivotal mechanism of various lifespan extending intervention (especially those that are based on environmental signals). Numerous lifespan extending interventions converge on autophagy (Melendez, et al., 2008). Enhanced autophagy is associated with human longevity and serum levels of the autophagy biomarker beclin-1 are increased in healthy centenarians (Emanuele, et al., 2014).

2.4.6.5 Chaperone

Although gerontogenes are associated with exhibiting chaperone binding [Figure 7 C. elegans Ageing Gene Network] ageing-suppressor genes are significantly associated with a wide spectrum of chaperone-related entities/activities like iron chaperone activity [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network, [Figure 10 Common Ageing Gene Network], metallochaperone activity [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 8 S. cerevisiae Ageing Gene Network; [Figure 10: Common Ageing Gene Network], endoplasmic reticulum chaperone complex [Figure 7 C. elegans Ageing Gene Network], chaperone-mediated protein folding [Figure 6 D. melanogaster Ageing Gene Network], chaperone-mediated protein complex assembly [Figure 8 S. cerevisiae Ageing Gene Network], regulation of chaperone-mediated protein complex assembly [Figure 5 M. musculus Ageing Gene Network], and positive regulation of chaperone-mediated protein complex assembly [Figure 5 M. musculus Ageing Gene Network]. Molecular chaperones influence lifespan as part of the stress response machinery (Walker & Lithgow, 2003).

Lifespan extension by reduction of translation may be due to a decreased load of misfolded and damaged proteins, which are primarily eliminated by chaperones and proteases. Reduced translation results in spare chaperone and proteolytic functions which can easily deal with the modified proteins (Hipkiss, 2007).

2.4.6.6 Proteasome

Ageing-suppressor genes, but not gerontogenes are commonly significantly associated with proteasome (proteasome complex, proteasome assembly, proteasome regulatory particle base subcomplex, proteasomal protein catabolic process, and proteasome-mediated ubiquitin-dependent protein catabolic process [Figure 10 Common Ageing Gene Network]. Elevated proteasome activity extends lifespan (Yao, et al., 2015).

Murine ageing genes are most significantly associated with Ubc [Figure 5 M. musculus Ageing Gene Network], which encodes ubiquitin C and participates in ubiquitin homeostasis. Human ageing genes also significantly associate with physical interactions to UBC [Figure 4 H. sapiens Ageing Gene Network]. Ubc is significantly downregulated during ageing in rat liver (Chen, et al., 2006). UBC was found as pivotal protein to interact with diverse Alzheimer's disease-associated pathophysiological molecular factors and which implies the reduced ubiquitin proteasome degradation system as one of the causative factors of Alzheimer's disease (Manavalan, et al., 2013).

Yeast ageing genes most significantly physical interact with RKR1, encoding a ubiquitin-protein ligase involved in chromatin organization, chromatin silencing at telomere, as well as ribosome-associated

ubiquitin-dependent protein catabolic process, and rescue of stalled ribosomes. RKR1 is already a known ageing-suppressor gene.

Although maintaining protein homeostasis is crucial for life and health, which type of autophagy and ubiquitin-proteasome system is most limiting the lifespan is unclear. Nucleophagy and mitophagy are commonly associated with ageing-suppressor genes. Moreover, in the organism with the highest number of known ageing genes, ageing-suppressor genes are associated with piecemeal microautophagy of nucleus and the nuclear proteasome complex [Figure 8 S. cerevisiae Ageing Gene Network]. Those subsystems have not yet been investigated in context of ageing much. However, it was observed that the nuclear pore complexes which are composed of long-lived proteins (i.e. proteins with low turnover) deteriorate in an age-dependent manner and cause loss of nuclear integrity and result in increased uncontrolled permeability (i.e. leakage) especially in postmitotic cells (D Angelo, et al., 2009). Proteolysis of parts of the nucleus could counteract this deterioration.

2.4.7 SUMOylation

Ageing-suppressor genes were also associated with protein SUMOylation [Human]. SUMOylation is basically a reversible post-translational modification that conjugates small peptide SUMO (small ubiquitin-like modifier) to a larger target protein.

The global protein SUMOylation and expression of components involved in SUMOylation are altered during the ageing process. Also key factors that control cellular senescence are known to be targeted by SUMOylation (Gong, et al., 2016).

2.4.8 Apoptosis

Gerontogenes are commonly significantly associated with cell death, programmed cell death, regulation of cell death, regulation of programmed cell death, positive regulation of cell death, positive regulation of programmed cell death [Figure 10 Common Ageing Gene Network]. Ageing-suppressor genes are also commonly significantly associated with all those, but additionally also commonly significantly associated with ectopic germ cell programmed cell death, programmed cell death involved in cell development, negative regulation of cell death, negative regulation of programmed cell death, negative regulation of oxidative stress-induced cell death [Figure 10 Common Ageing Gene Network].

Therefore, it appears that ageing-suppressor genes prevent cell death especially cell death that is induced by stress. However, gerontogenes are commonly significantly associated with negative regulation of extrinsic apoptotic signalling pathway [Figure 10 Common Ageing Gene Network].

Ageing enhances apoptosis and susceptibility to apoptosis in several types of intact cells, while ageing suppresses the apoptosis of damaged, malignant and, senescence cells (Higami & Shimokawa, 2000). The increased resistance to apoptosis enhances the ageing process (Salminen, et al., 2011).

Gerontogenes are also significantly associated specifically with positive regulation of neuron death which might be related to the occurrence of neurodegenerative disease like Alzheimer's disease and Parkinson disease or the general decline in cognitive functionalities during increasing age [Figure 4 H. sapiens Ageing Gene Network].

2.4.9 Cellular Senescence

In mammals ageing genes are significantly involved in cellular senescence including replicative and (oxidative) stress-induced premature senescence as well as regulation of cellular senescence (positive and negative regulation) [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network]. Ageing-suppressor genes are commonly associated with senescence, cellular senescence, replicative senescence, regulation of cellular senescence and in particular negative regulation of cellular senescence [Figure 10 Common Ageing Gene Network].

Interestingly, while ageing-suppressor genes are involved in negative regulation of cellular senescence, gerontogenes are involved in positive regulation of cellular senescence [Figure 4 H. sapiens Ageing Gene

Network; Figure 5 M. musculus Ageing Gene Network]. This makes perfect sense considering that cellular senescence is a kind of ageing hallmark in mammalian organisms. Preventing or eliminating senescence cells is usually associated with lifespan extension. Interventions like dietary restriction are known to prevent the accumulation of senescence cells (Wang, et al., 2010; Liu, et al., 2012). Further, genetic or pharmacological elimination of senescence cells in mice is capable of lifespan extension (Baker, et al., 2016; Zhu, et al., 2015; Zhu, et al., 2016).

2.4.10 Hormones

2.4.10.1 Insulin/IGF-1

Gerontogenes were found to be associated with insulin/insulin-like growth factor signalling (including insulin binding, insulin receptor substrate binding, insulin receptor binding, insulin receptor complex, cellular response to insulin stimulus, insulin-activated receptor activity, and regulation of insulin-like growth factor receptor signalling pathway) while ageing-suppressor genes in particular were found to be associated with negative regulation of this specific type of signalling (including negative regulation of insulin secretion involved in cellular response to glucose stimulus) [Figure 5 M. musculus Ageing Gene Network; Figure 9 Common Ageing Gene Network]. This confirms the observations that insulin/insulin-like growth factor signalling is a control circuit of ageing and that its enhancement result in lifespan shortening and its suppression is resulting in lifespan extension. Dietary restriction is usually correlated with reduced insulin/insulin-like growth factor signalling as well (Chiba, et al., 2007; Bartke, 2008).

The evolutionary conserved insulin and insulin-like growth factor signalling (IIS) has a crucial role in control of longevity. Studies on the genetic and metabolic characteristics that are associated with healthy longevity and old age indicate that the conserved and ancient IIS also participates in human lifespan control (van Heemst, 2010).

2.4.10.2 Somatotropin

Gerontogenes are also associated with somatotropin secreting cell development and differentiation [Figure 5 M. musculus Ageing Gene Network] which with the former is the cellular development process whose specific outcome is the progression of a somatotropin secreting cell over time. A somatotropin secreting cell produces growth hormone (a.k.a. somatotropin).

Gerontogenes are associated with positive regulation of growth hormone secretion, while ageing-suppressor genes are associated with negative regulation of growth hormone secretion [Figure 5 M. musculus Ageing Gene Network]. Growth hormone seems to mediate ageing in mammals in a systemic fashion (Hill, et al., 2016). Human dwarfs (dwarfism) with growth hormone deficiency tend to live longer and stay free from many age-related disease like cancer and diabetes. Dietary restriction lowers somatotropin levels as well (Bartke, 2012).

2.4.11 Mitochondrial Dysfunction

2.4.11.1 Mitochondria

Gerontogenes are commonly significantly associated with mitochondrial DNA metabolic process and mitochondrial respiratory chain complex III biogenesis. Ageing-suppressor genes are associated with mitochondrial nucleoid, organization, and disassembly [Figure 9 Common Ageing Gene Network]. Further gerontogenes have significant associations with negative regulation of establishment of protein localization to mitochondrion, mitochondrial fission, and ATP biosynthetic process [Figure 9 Common Ageing Gene Network].

More specifically gerontogenes are significantly associated with negative regulation of mitochondrion organization, while ageing-suppressor genes are commonly significantly associated with positive regulation of mitochondrion organization [Figure 9 Common Ageing Gene Network]. This may indicate that gerontogenes participate in mitochondrial dysfunction and ageing-suppressor genes counteract it. Mitochondrial dysfunction and decreased mitochondrial biogenesis are believed to participate in metabolic

abnormalities and loss of organ function, which will eventually contribute to ageing and decreases lifespan (Tocchi, et al., 2015).

2.4.11.2 Reactive Oxygen Species

According to the free radical theory of ageing which was a rather recent theory of ageing more than 30 years ago (Koster, 1986), free radicals (that are produced as by-products of metabolism) are driving ageing by damaging biomolecules. There are a variety of types of radicals, but the predominant ones in biological system are derived from oxygen and collectively called reactive oxygen species. A radical is an atom or molecule that has one or more unpaired electrons. Radicals are formed as necessary intermediates in a variety of biochemical reactions and also function as signalling molecules, but when generated in excess or not appropriately controlled, free radicals can wreak havoc on a wide range of macromolecules.

Gerontogenes are significantly and commonly associated with regulation of reactive oxygen species metabolic process [Figure 9 Common Ageing Gene Network], especially, positive regulation of reactive oxygen species metabolic process [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 9 Common Ageing Gene Network]. Ageing-suppressor genes were found to be associated with negative regulation of reactive oxygen species metabolic process and removal of superoxide radicals, in particular positive regulation of removal of superoxide radicals as well as oxidoreductase activity acting on superoxide radicals as acceptor [Figure 5 M. musculus Ageing Gene Network; Figure 9 Common Ageing Gene Network].

2.4.12 Regeneration

Ageing-suppressor genes are associated with regeneration [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 9 Common Ageing Gene Network], specifically of liver [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network], [Figure 9 Common Ageing Gene Network], neuron [Figure 5 M. musculus Ageing Gene Network], [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network, [Figure 9 Common Ageing Gene Network], dendrite [Figure 6 D. melanogaster Ageing Gene Network], axon [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 9 Common Ageing Gene Network] as well as myoblast differentiation involved in skeletal muscle regeneration [Figure 5 M. musculus Ageing Gene Network]. Regeneration is the growth of anew or lost tissue or destroyed parts of organs.

The regrowth of a lost or destroyed body parts, such as an organ or tissue is vital for tissue/organ maintenance. This process may occur via renewal, repair, and/or growth alone. Regeneration decelerates with age. Long lived or immortal organisms often have an enormous regenerative potential which is mediated by Wnt signalling (Galliot & Chera, 2010; Heber-Katz, et al., 2006; Lengfeld, et al., 2009).

2.4.12.1 Stem Cells

Ageing genes have significant associations with stem cells. In particular ageing-suppressor genes are associated with positive regulation of stem cell proliferation [Figure 5 M. musculus Ageing Gene Network; Figure 4 H. sapiens Ageing Gene Network], and negative regulation of stem cell differentiation [Figure 5 M. musculus Ageing Gene Network]. Further, ageing-suppressor genes are associated with stem cell division, germ-line stem cell population maintenance, and intestinal stem cell homeostasis [Figure 6: D. melanogaster Ageing Gene Network]. It seems like that promoting stem cell proliferation and preventing their loss from differentiation is a mechanism in which ageing-suppressor genes can counteract organismal ageing.

Similarly ageing-suppressor genes are significantly associated with hematopoietic stem cell proliferation and differentiation [Figure 5 M. musculus Ageing Gene Network] in particular negative regulation of hematopoietic stem cell differentiation [Figure 4 H. sapiens Ageing Gene Network]. Hematopoietic stem cells are multipotent stem cells that replenish the body with new blood cells. Having a greater reservoir of hematopoietic stem cells would provide the organism a higher capacity to renew its blood. Hematopoietic

stem cells are themselves ageing, so generating new hematopoietic stem cells through differentiation into this lineage would enhance this stem cell population (Geiger, et al., 2005).

Adult or tissue-specific stem cells are not immortal. Their efficiency is limited by natural ageing in common with most other somatic cell types (Nurkovic, et al., 2016). There cannot be anything related to stem cells in yeast as a single-cell organism, but replicative cell ageing is assumed to be a model of stem cell ageing and is also more strongly associated with ageing-suppressor genes [Figure 8 S. cerevisiae Ageing Gene Network].

2.4.12.2 Wound Healing

Ageing genes are significantly associated with wound healing [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network]. Ageing-suppressor genes are significantly associated with wound healing including wound healing spreading of epidermal cells, while gerontogenes are associated with negative regulation of vascular wound healing [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network].

With higher age wound healing is delayed (Pittman, 2007). Enhancing wound healing might expect an organism to increase its capability to live longer.

2.4.13 Circadian Rhythm

Ageing genes are associated with circadian rhythm, gerontogenes and ageing-suppressor genes specifically as well. Gerontogenes are associated with entrainment of circadian clock by photoperiod and circadian behaviour. Ageing-suppressor genes on the other side are associated with regulation of circadian rhythm, positive regulation of circadian rhythm, and circadian regulation of gene expression and translation [Figure 6 D. melanogaster Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network].

There are well known age-related effects on circadian rhythms including reductions of rhythm amplitude, alterations in re-entrainment and increased fragmentation (Pang, et al., 2004). Lifespan extending interventions like dietary restriction are known to reset the circadian clock and make circadian rhythmicity more robust (Froy, et al., 2008).

2.4.14 Cytoskeleton

Both gerontogenes and ageing-suppressor genes are commonly significantly associated with cytoskeleton organization and cytoskeleton-dependent intracellular transport. Ageing-suppressor genes are specifically associated with regulation of actin cytoskeleton organization, actin cytoskeleton organization, microtubule cytoskeleton organization, actin cytoskeleton reorganization, and establishment or maintenance of cytoskeleton polarity [Figure 6 D. melanogaster Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Cellular ageing is characterized by the appearance of various cell dysfunctions. However the possible involvement of the microtubules in the ageing process has no yet been extensively investigated (Raes, 1991).

atl is the most significant association based on physical interaction of fly ageing genes. atl encodes atlastin, a GTPase homologous to mammalian ATL2, that is involved in endoplasmic reticulum membrane fusion and microtubule depolymerisation. atl is also significantly associated with fly gerontogenes. Loss of atlastin induces age-dependent death of dopaminergic neurons in *Drosophila*. atl null flies are paralysed by mechanical shock such as bumping or vortexing and exhibit degeneration of dopaminergic neurons (Lee, et al., 2008). atl and spastin mutants display similar adult phenotypes including short lifespan, impairments in locomotor activity, and age-dependent neurodegeneration (Lee, et al., 2009). Note that atl nor spastin were yet classified as ageing-suppressor genes, nor ageing genes. Hence this is a *in silico* validation of a candidate.

The most significant physical interaction partner of worm ageing genes is *atn-1* which encodes an alpha-actinin homolog [Worm]. *atn-1* exhibits actin binding and involved in mitotic nuclear division.

2.4.15 Immunosenescence

Ageing genes are involved in different aspects of the immune system. Ageing-suppressor genes are significantly associated with humoral immune response mediated by circulating immunoglobulin [Figure 4 H. sapiens Ageing Gene Network], and somatic recombination of immunoglobulin genes [Figure 5 M. musculus Ageing Gene Network], regulation of cytokine secretion [Figure 5 M. musculus Ageing Gene Network]. Gerontogenes are significantly associated with immune system process [Figure 7 C. elegans Ageing Gene Network], regulation of innate immune response [Figure 7 C. elegans Ageing Gene Network] as well as positive regulation of immune response [Figure 6 D. melanogaster Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network] and prostaglandin secretion involved in immune response [Figure 5 M. musculus Ageing Gene Network].

Ageing genes have clear and significant associations with inflammation and its regulation. Gerontogenes are directly significantly associated with inflammatory response, including positive regulation of acute inflammatory response, positive regulation of leukotriene production involved in inflammatory response [Figure 5 M. musculus Ageing Gene Network]. Ageing-suppressor genes are significantly associated with regulation of cytokine production involved in inflammatory response [Figure 5 M. musculus Ageing Gene Network], negative regulation of inflammatory response [Figure 5 M. musculus Ageing Gene Network], negative regulation of acute inflammatory response [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network], negative regulation of respiratory burst involved in inflammatory response [Figure 4 H. sapiens Ageing Gene Network].

Ageing of the immune organs and hence deterioration of the immune system promotes systematic inflammation and age-related disease (including autoimmune diseases and cancer) as well as reduced responsiveness to vaccination and increased susceptibility to infections (Sidler, et al., 2013; Chen, et al., 2014). When the immune organs developmentally driven degenerate (e.g. mammalian thymus) it mounts strong systematic inflammation (Chen, et al., 2014). Immunosenescence is caused by age-specific changes in the nuclear lamin expression which contributes to heterochromatin loss of genes involved in the immune response (Chen, et al., 2014). Altered gene expression, DNA and histone H3K9 hypomethylation, increased genome instability and apoptosis are observed in the primary and secondary immune system organs of aged organisms. Alterations in gene expression and epigenetic regulation occur already in early ages (Sidler, et al., 2013).

With increasing age tissue become more and more inflamed. While acute inflammation is good in response to injury and infection, chronic inflammation has negative effects. This phenomenon is so predominant that it got its own name, *inflammageing*. By their very activity gerontogenes could lead to inflammageing (Franceschi, et al., 2007).

2.4.16 Nucleus

Gerontogenes and ageing-suppressor genes have both significant associations with the nucleus. The nucleus is like the central processing unit of the cell.

The nuclear matrix is mostly significantly associated with ageing-suppressor genes as a location [Figure 4 H. sapiens Ageing Gene Network; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 10 Common Ageing Gene Network]. The nuclear matrix is poised to play a critical role in the ageing process as it is important for DNA organization and nuclear structural morphology. The nucleus increases in size and becomes more round with age. There appear to be quantitative (but not qualitative) alterations in the prominent nuclear matrix proteins with age (Pienta, et al., 1992). Thus ageing-suppressor genes could counteract these trends by stabilizing the nuclear matrix.

Beyond this, the mitochondria-nucleus signalling pathway is significantly associated with ageing-suppressor genes [Figure 8 S. cerevisiae Ageing Gene Network]. Dysfunctional mitochondria signal to the nucleus to induce the expression of a set of genes (retrograde regulation/response) that increases lifespan (Osiewacz & Kimpel, 1999).

Ageing-suppressor genes are associated with (late) nucleophagy [Figure 8 S. cerevisiae Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network], piecemeal microautophagy of nucleus [Figure 8 S. cerevisiae Ageing Gene Network], nuclear ubiquitin-proteasome system and nuclear proteasome complex

[Figure 8 S. cerevisiae Ageing Gene Network]. Among the different proteasomal systems, the nuclear proteasomes are affected most profoundly by ageing possibly triggering significant changes in cellular signalling and transcription (Rodriguez, et al., 2010).

2.4.17 Growth and Development

The regulation of growth rate is yet another process controlled by ageing genes. Ageing genes have significant associations related to different scales of growth. Gerontogenes alone are significantly and commonly associated with regulation of cell growth and organ growth including positive regulation of cell and multicellular organism growth [Figure 9 Common Ageing Gene Network]. Gerontogenes are clearly involved in positive regulation of growth processes, while ageing-suppressor genes have a tendency to be involved in negative regulation of growth [Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network; Figure 9 Common Ageing Gene Network]. Growth is tightly related to ageing, as most organisms stop growing after reaching maturation and start to age. While growth is not so important in an adult organism, those genes that initially functioned to drive growth and are essential for development may cause havoc in the adult. Interventions that slow down growth such as dietary restriction also increase lifespan. Therefore there exists an inverse relationship between growth and ageing. A phenomenon known as antagonistic pleiotropy.

Besides controlling growth, gerontogenes are significantly associated with development [Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network; Figure 7 C. elegans Ageing Gene Network Figure 10 Common Ageing Gene Network]. Both ageing-suppressor genes and gerontogenes are associated commonly to development, but gerontogenes are commonly and strongly associated to multicellular organism development, embryo development, epidermis development, neuron projection development, system development, and developmental maturation [Figure 10 Common Ageing Gene Network].

The most significant candidate physically interacting with worm gerontogenes is *pal-1* which encodes a transcriptional regulator involved in development. *pal-1* also genetically interacts significantly with gerontogenes. Based on those associations, *pal-1* might be a gerontogene as well.

Ageing could be regarded as an extension of development. As such, ageing could be mediated by the same genes that mediate development as both are unfolding over time. This could also be another example of antagonistic pleiotropy where changes that are beneficial early in life (i.e. during development) become detrimental later in life (i.e. during ageing) (Ungewitter & Scrable, 2009).

Ageing genes or genes that prolong or antagonize longevity have been studied collectively for common associations (Kwon, et al., 2010; Fernandes, et al., 2016). However those studies mostly found broad associations and have not identified specific associations with defined ageing classes like gerontogenes and ageing-suppressor genes and pinpointed to causal relations in interpretations.

Previous investigations in associations of genes that enhance or suppress the ageing process found p53 signalling, cell cycle, hypoxia response via HIF activation, regulation of autophagy and oxidative phosphorylation associated with ageing-suppressors. Gerontogenes were found to be associated with insulin, IGF1 and growth hormone signalling, mTOR signalling and ribosomes (Fernandes, et al., 2016). However these investigations did not yet discuss much about precise regulations and specific terms as done here comprehensively. Regulation of p53 as well as p53 controlled genes have been found here to be associated with both ageing-suppressor genes and gerontogenes [Common Associations with Ageing Genes Across Species]. p53 actually refers to a family of closely related transcription factors consisting (in mammals) of p53, p63 and p73, each of which has various isoforms with very distinct effects. It has homologs in invertebrates and maybe has distant homologs in single cellular organisms. p53 itself has been assumed to be an oncogene, but also functions as tumour-suppressor (Baker, et al., 1989). Similar p53 has been repeatedly found to have both ageing-suppressing as well gerontogenic effects. Regulation of cell cycle is associated with ageing-suppressor genes and gerontogenes. Gerontogenes are significantly associated with mitotic cell cycle, while ageing-suppressor genes are significantly associated with meiotic cell cycle [Common Associations with Ageing Genes Across Species]. HIF-1 signalling pathway is commonly associated with ageing-suppressor genes [Common Associations with Ageing

Genes Across Species]. Ageing-suppressor genes and gerontogenes both are commonly associated with oxidative phosphorylation, but regulation of oxidative phosphorylation is associated with gerontogenes, while oxidative phosphorylation uncoupler activity is associated with ageing-suppressor genes [Common Associations with Ageing Genes Across Species]. Insulin/insulin-like growth factor and growth hormone signalling have been all been strongly associated with gerontogenes, while ageing-suppressor genes have been found to be involved in their negative regulation [Hormone]. Ageing-suppressor genes have been found here to be positive and gerontogenes to be negative regulators of different forms of autophagy, where ageing-suppressor genes have been found to be associated with various forms of autophagy itself [Autophagy]. Gerontogenes were found here to be strongly associated with TOR signalling and its positive regulation, while ageing-suppressor were found to be associated with its negative regulation [TOR]. Gerontogenes were identified here to be associated with ribosome and its structural constituents [Common Associations with Ageing Genes Across Species] as well as its positive regulation of ribosomal biogenesis [Fly] whereas ageing-suppressor genes were found to be associated with negative regulation of ribosomal biogenesis [Mouse].

Guilt-by-association based on the interactome can predict novel ageing genes as well as processes, functions and location associated with the ageing process. This was validated by showing that already known ageing genes are rediscovered (i.e. are among the most significant genes). Other network topological measures such as betweenness and shortest path can be used as well to identify unknown ageing genes as well as processes, functions, and locations crucial for lifespan control.

Using defined classes of ageing genes as input for the guilt-by-association applied on the interactome often resulted in different genes being most significant, though others remain very significant for both subclasses. It is tempting to speculate that gerontogenes are associated with different genes (complexes, pathways, etc.) than ageing-suppressor genes. It is at least suspected that different kind of processes can promote ageing or suppress ageing. However gerontogenes and ageing-suppressor genes can also be simple activators and repressors of the very same complexes, pathways and processes and *vice versa*. The directionality of regulation identified here strongly support this.

While several genes, processes, activities, locations have been associated with ageing genes, why they might be important in ageing is not yet inferred. Further whether those play any causal role or are more effectors is not distinguished. Regulatory networks could be employed to infer causality, e.g. transcription factors and post-translational modifying proteins to some extent. Moreover, a class of genes associated with longevity (longevity-associated genes) derived from genomic variants associated with centenarians and supercentenarians could be characterized as well as genes known to be associated with cellular senescence. In yeast ageing genes could be subdivided to whether they are impacting on replicative or chronological lifespan. It is not certain to which extent those two types of ageing are overlapping and whether genes that affect replicative lifespan also affect chronological lifespan and *vice versa*. It could be tested whether replicative ageing genes tend to be also chronological ageing genes and the other way around as well.

The processes, functions (activities) and locations (compartments) that were found to be common to the known ageing genes and defined subclasses in different species might reveal common mechanisms of ageing.

2.5 Conclusion

Ageing is under control of genes. Some genes promote ageing (gerontogenes) while other counteract it (ageing-suppressor genes). Guilt-by-association has been used to identify new candidate genes and associated processes, activities and locations of specific ageing gene subclasses. Gerontogenes and ageing-suppressor genes are both commonly associated with certain processes, activities and locations across species boundaries. Those two classes have quite frequently antagonizing associations. Gerontogenes are commonly associated with positive regulation of TOR, inflammation, translation, apoptosis, cellular senescence, growth and development, as well as negative regulation of telomere maintenance and proteostasis. Ageing-suppressor genes are associated with DNA metabolism (including DNA repair and telomere maintenance), epigenetics (including DNA methylation), stress response, regeneration, circadian clock, cytoskeleton, MAPK, and as well as negative regulation of cellular senescence and mitochondria dysfunction.

3 Ageing Signatures

Abstract: Ageing is accompanied by profound and discrete changes in gene expression. Gene expression profiles provide a wealth of data to give insights into the molecular mechanism of ageing. Transcriptional consensus signatures of ageing in humans and common biomedical model organisms were established as well as consensus signatures of accelerated ageing and cellular senescence in humans. Ageing upregulates transcription regulation, TOR signalling, apoptosis and inflammation and downregulates Wnt signalling, epigenetics, and proteostasis. Accelerated ageing upregulates Notch signalling, and downregulates Wnt signalling. Cellular senescence, just as seen for whole organism ageing, is associated with an upregulation of inflammation, but also negative regulation of cell cycle and apoptosis.

3.1 Background

Several genes have been identified that, when mutated, can reduce and/or extend lifespan [Ageing Genes]. However much about ageing remains unknown and to be explained. New technologies allow the simultaneous assay of expression levels of thousands of genes. These can be explored to answer the questions of how and why ageing might occur. With the right experimental design and computational analysis, differential gene expression between different ages can be obtained with high confidence. The possibility to profile the entire genome in an unbiased way is a great asset for the study of complex biological phenomenon such as ageing.

Ageing involves the changes in multiple genes involving multiple processes, activities, and locations, some of which may not yet be identified but can have a very important role in modulating the ageing process. Gene expression profiling of wild type (i.e. normal) ageing, and of mutants with accelerated ageing or increased lifespan may provide insights into potential mechanisms of how ageing operates in organisms [Molecular Profiling to Decipher Ageing].

During ageing there are myriads of changes that occur on different scales. From whole physiological (phenotypes that can be observed) down to the molecular level. The level of expression of genes changes with increasing age in a way that can be assessed via molecular profiling. Ageing gene expression changes are measured in time series. Hence time-course experiments are necessary in the study of ageing. Therefore ageing gene expression datasets are basically time series datasets. In order to enable the identification of age-related trends, studies of the ageing process should examine at least three ages (young, middle-aged, and old) and ideally many more than this. Choosing only two ages (young vs. old) risks missing trends that may occur during the lifespan; a peak in midlife followed by a decline to near youthful levels, for instance, would be missed by an experimental design or computational analysis including only two ages (Golden & Melov, 2007). If samples of various ages are measured as usual in studies of ageing in humans it is not obvious what to compare. However one can put together samples (from individuals with a certain age) into groups to represent certain periods of age. To derive ageing gene expression molecular signatures one usually compares young versus old, young vs. middle-aged, and middle-aged vs. old.

Extensive studies in model organisms and humans have identified many genes that exhibit age-dependent expression changes. In addition, individuals with progeria (accelerated ageing) and in particular Hutchinson-Gilford progeria syndrome (HGPS) have been profiled. There also exist time series gene expression profiles of cellular senescence (Kim, et al., 2013) and centenarians.

However, for analysing ageing gene expression data a major difficulty is to define young, middle-aged and old groups. In humans young (20-30 years), middle-aged (45-55 years), and old (>65 years) were defined (Nair, 2005). Though this would leave gaps at the ages of 0-20, 30-45 and 55-65 years. In human everything under 20 years is considered to be developmental, i.e. 0-20 years is considered to be the

development phase in humans (Somel, et al., 2010). In a somewhat different classification, young was defined as 15-30 years, middle-aged as 40-55 years and old as 60+ years (Aliper, et al., 2015). In yet another classification, young was considered to be from 18-30 years while old as 59-76 years (Lanza, et al., 2008). Finally another classification without any gaps was defined with human development from 0-20 years, young adulthood from 20-40, middle-aged from 40-60 and old age from 60 onwards (Kang, et al., 2011).

For model organism the subdivision into life stages is similar difficult and ill-defined. Monkey under 5 years (< 5 years) are considered developing, from 5 to 8 young, middle-aged in the range of 8-12 years, and old if aged over 12 years. Rat can be defined to be young up to 5 months, middle-aged beyond 11 months and old between 25-29 months (Takahashi, et al., 1987). Mice are mature adults with 3-6 months, middle-aged between 10-15 months and old between 18-24 months (Flurkey, et al., 2007). Another schema defines young (4 month-old), middle-aged (13 month-old), and old (25 month-old) mice (Karelus & Nelson, 1992). A definition is to declare young mice aged less than 6 months, middle aged mice are aged 6-17 months and old mice are more than 24 months old (Lesne, et al., 2006). Fruit fly were defined as young (7 days old), middle-aged (30 days old), and old (50 days old) (Brigui, et al., 1990). In nematode, two-day-old adult worms were defined as young adults, six-day old adult worms as middle-aged adults, and 10-day old adults worms as aged adults (Guha, et al., 2014). In yeast a replicative lifespan of 9 generations was considered as middle-aged and 13.5 generations as old age (Steinkraus, et al., 2008). Gaps in age range definition can be accommodated by interpolating their boundaries.

Based on this, life stages and phases [Table 1: Life Stages and Phases] as well as age ranges for humans and common biomedical model organisms were defined [Table 2: Age Ranges].

Table 1: Life Stages and Phases. Subdivision of the life into stages and phases.

Life Stage	Phase
in uture	Development
Juvenile	
Adolescent	
Young Adult	Adulthood
Middle-Aged Adult	
Old Adult	

Table 2: Age Ranges. Ranges of ages for specific life stages (Fox, et al., 2006).

Species	Development	Young	Middle-Aged	Old	Unit
Human	0 - 20	20 - 40	40 - 60	> 60	Years
Monkey	0 - 5	5 - 8	8 - 12	> 12	Years
Rat	0 - 5	5 - 11	11 - 25	> 25	Months
Mouse	0 - 3	3 - 6	6 - 14	> 14	Months
Fly	0 - 7	7 - 30	30 - 50	> 50	Days
Worm	0 - 2	2 - 6	6 - 10	> 10	Days
Yeast	0 - 2	2 - 9	9 - 13	> 14	Generations

DNA microarray and RNA-seq based profiling represent effective methods to analyse cellular or tissue-specific gene expression on the genome-level as well as prioritize gene candidates (Anand, et al., 2015). Ageing has been transcriptional profiled in various organisms including human, monkey, rat, mouse, horse, fish, fly, nematode, and yeast among others. Here it will be focused primarily on human and

common biomedical model organisms for which significant gene expression profiles of various ages exist including young and old.

Tissue and age contribute more to the global differences in gene expression than do sex, ethnicity and inter-individual variation (Kang, et al., 2011). High-throughput gene expression profiles of ageing typically result in differential expression of hundreds or thousands of genes. To allow for the interpretation of these changes, sets of genes can be identified that have some common characteristics. This is often referred to as gene set enrichment, where statistical tests are applied to the molecular signature from those profiles to identify significant enrichment of groups of genes in these signatures.

The enrichment can be quantitatively measured by some common statistical methods including Chi-square, Fisher's exact test, Binomial probability, and Hypergeometric distribution (Huang, et al., 2009). The Fisher Exact Test is a usual method used for gene set enrichment analysis. However, there are modifications of the Fisher Exact Test available such as the EASE score that allow for the ranking of biological categories associated with sets of genes from gene list based on the co-occurrence/enrichment of the category with the gene list (Hosack, et al., 2003).

Enrichment analysis tools can be grouped into at least three different classes based on the difference in algorithms (Huang, et al., 2009):

1. Singular Enrichment Analysis
2. Gene Set Enrichment Analysis
3. Modular Enrichment Analysis

Singular enrichment analysis takes preselected genes from a signature by some common metrics (like t-test p-value < 0.05 and fold change > 1.5) and iteratively tests the enrichment of each annotation term one-by-one in a linear mode. Gene set enrichment analysis in contrast is a "no-cutoff" strategy that takes all genes from the signature without selecting significant genes. It therefore 1) reduces the arbitrary factors and 2) uses all information obtained from profiling. Modular enrichment analysis inherits the enrichment calculation of singular enrichment analysis and incorporates the term-to-term relationships. The independence assumption of singular enrichment analysis is seriously violated by extensive correlation between genes (a well recognised phenomenon) (Gatti, et al., 2010). Gene set analysis can be extended to longitudinal data in order to allow time-course gene set analysis (Hejblum, et al., 2015).

3.2 Methods

Gene expression series, samples, datasets, and platforms (automatically downloaded from the Gene Expression Omnibus File Transfer Protocol server) were parsed. Lines with null/NaN values were discarded. Intensity values were interpolated from missing values via mean. All values were log2-transformed and quantile normalized if necessary (i.e. if not done yet). Individual molecular profiles were derived from multiple samples within a series [Molecular Profiling to Decipher Ageing]. Platform files were used to obtain annotation data for the profiles (e.g. sequence identifiers and gene symbols).

The precise definition of a molecular signature is provided in the following which is supported by [Figure 13 Definition of a Molecular Signature]. A molecular signature is derived from RNA, protein, or metabolite expression profiles and is indicative of a biological transition (e.g. from healthy to diseased or from young to old). This pattern of expression difference is comprised of unique clusters of RNA, proteins, metabolites that display differential levels of expression. While a molecular expression profile is the measurement of a set of molecular entities representing a single biological state, a molecular signature represents the differences between such states (e.g. old versus young, diseased versus healthy, mutant versus wild-type, intervened versus unperturbed, etc.). A molecular signature can be represented in various mathematically formulations just like transcription factor binding motifs can be represented in different ways (e.g. regular expression, weight matrices, etc.). A molecular signature can be as simple as a set of genes (i.e. set theory) or more concrete as a vector where each components value represents the expression difference of a molecular entity such as a RNA. Thus a molecular signature can be a shortlist of most differentially expressed genes or a vector of fold changes in its most simple representations.

–

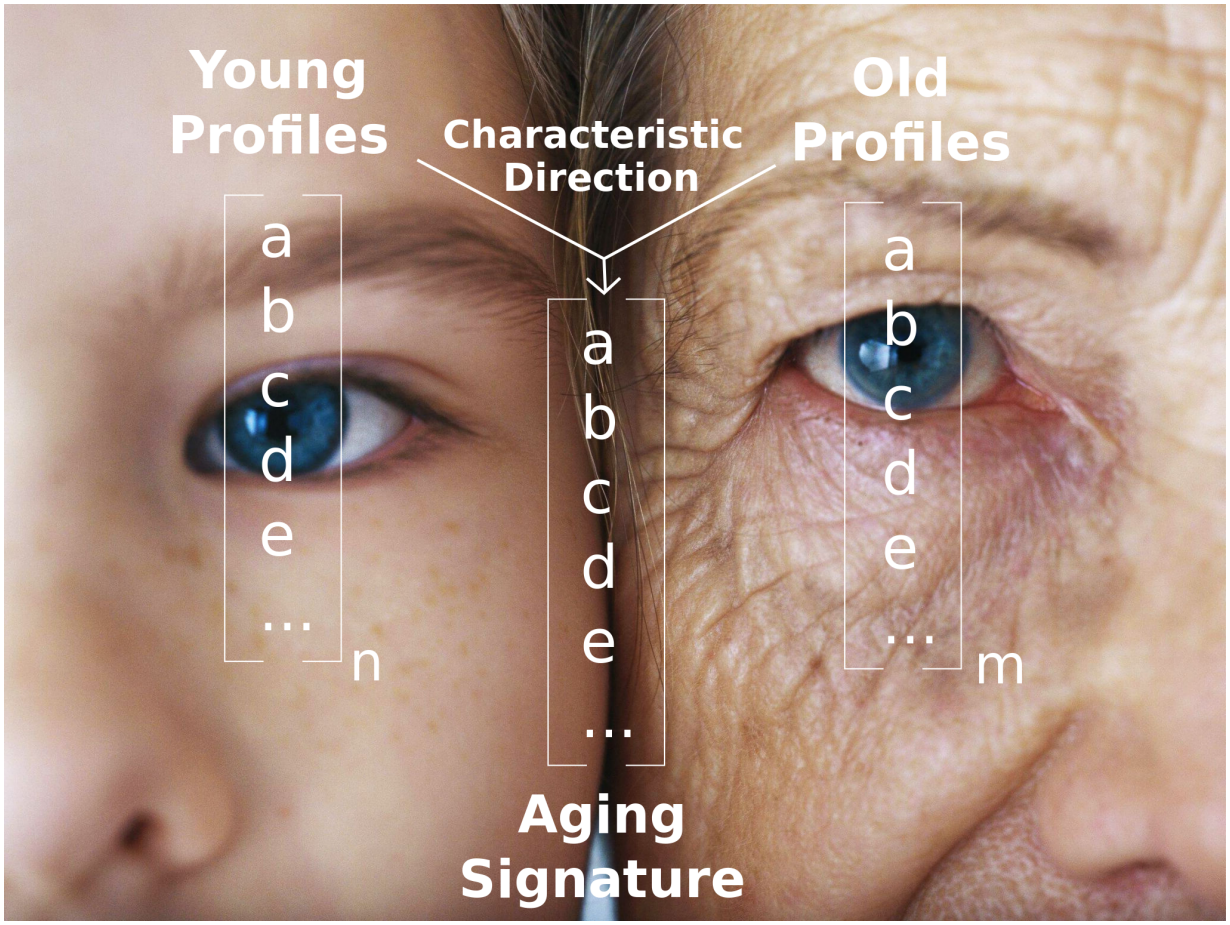

Figure 11: Definition of a Molecular Signature.

For each signature differential expression was calculated via the Characteristic Direction method which is superior to many standard methods of differential expression calculation (Clark & All, 2013; Clark, et al., 2014). The Characteristic Direction, a geometric approach to differential expression analysis, is a multivariate approach to determine differentionally expressed genes [Figure 12 Characteristic Direction]. The Characteristic Direction is better able to identify differential expressed genes than univariate approaches including: fold-change, SAM, Welch's t-test, LIMMA, and DESeq. Characteristic Direction uses a Linear Discriminant Analysis (LDA) classifier in which probability that a sample x derives from each of the classes G is modelled with the Bayes' rule by making assumption that class-conditional density is a multivariate Gaussian and covariance matrix for each class boundary is a hyperplane. Characteristic Direction naturally incorporates a regularization scheme to deal with the problem of the curse of dimensionality. The Characteristic Direction method provides an intuitive geometrical picture of differential expression in terms of a single direction. The square of each component is a measure of importance of corresponding gene in differential expression. Any gene expression profile can be represented as a point in this space. The direction can be represented by a unit vector. The relative magnitudes of components of vector correspond to the relative significance of genes in distinguishing between two biological conditions. Because direction is represented as a unit vector, the sum of squares of components is equal to unity. Each square of each component can be interpreted as being equal to fractional contribution of corresponding gene to the total difference between transcriptional profiles of two biological conditions. Therefore the Characteristic Direction can be used to rank genes and isolate the most relevance. The difference between samples can be characterized by a single vector. The magnitude of the vector quantifies the magnitude of the difference in expression between two groups of samples. The unit vector parallel to the Characteristic Direction quantifies contribution of each gene to the total difference. The

Characteristic Direction Method is more sensitive than t-test in recovering differentially expressed genes. It is also more pronounced when few samples are available for each condition.

Here the characteristics direction coefficients are calculated for each signature where samples are grouped so that the main discriminating factor is age. If sample size allows (at least more than 3) and gender annotation is provided gender specific signatures are calculated. The datasets used for deriving signatures can be requested from the author. Respective profiles (e.g. old vs. young) were used to create molecular signatures representing certain biological conditions, states, or processes (e.g. ageing). Young versus old, young versus middle-aged and middle-aged versus old were compared if possible and the samples can be grouped into those. In the case of a continuum of ages of samples, periods of around 1-6 years (in humans) were defined in such a way that each group has multiple replicates (2-3 or more).

Most dataset used can have several derived signatures (e.g. varying the gender or age ranges). Signatures for individual tissues were derived including female and male-specific signatures as well as gender- and tissue-independent consensus signatures. Signatures were generated common to multiple datasets (i.e. series) across cell types and tissues. For deriving a consensus signature the Characteristic Direction coefficients were summed up for each gene.

–

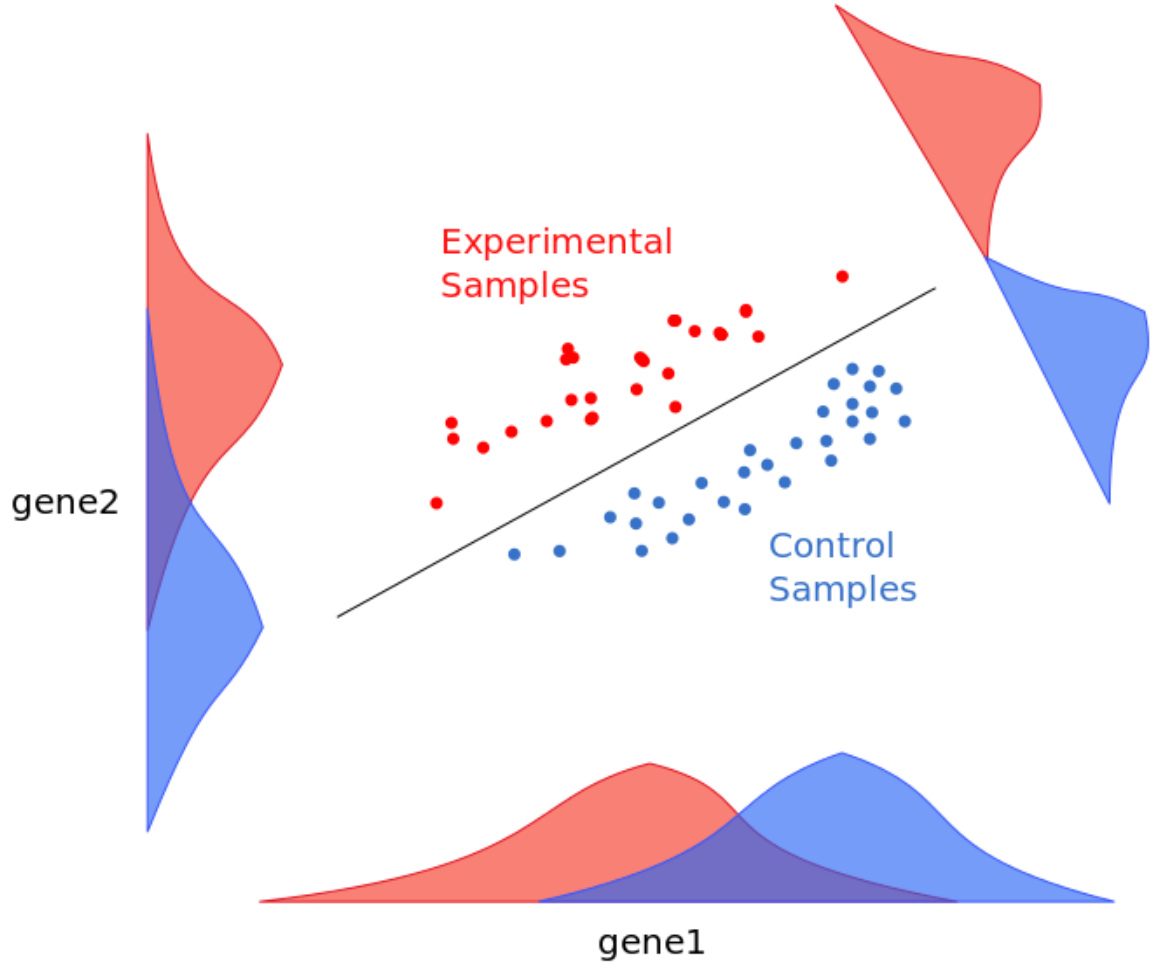

Figure 12: Characteristic Direction.

The Principal Angel Enrichment Analysis, a natural extension of the characteristic direction method, was used to assess the significance of associations with interactions of genes/proteins (derived from BioGRID Version 3.4.140) as well as processes, activities, locations (as defined in the Gene Ontology (Ashburner,

et al., 2000)) and chromosomes. p-values were adjusted according to Benjamini-Hochberg (Benjamini & HochBerg, 1995). q-values were combined via the Z-method (Whitlock, 2005).

The significant results of Principle Angle Enrichment Analysis is visualized as networks by connecting nodes via the shortest path algorithm and applying force-directed graph layout (Kobourov, 2012). Briefly, the significant nodes are visualized together as interaction networks. The significance to be associated with differentially expressed genes are used for the node size. For this q-values were converted to node size scale by adding to the minimum size of 5 the negative of the exponent of the q-value divided by two. Similar, nodes that are significantly associated with any gene classes were assigned the colour of the respective class.

–

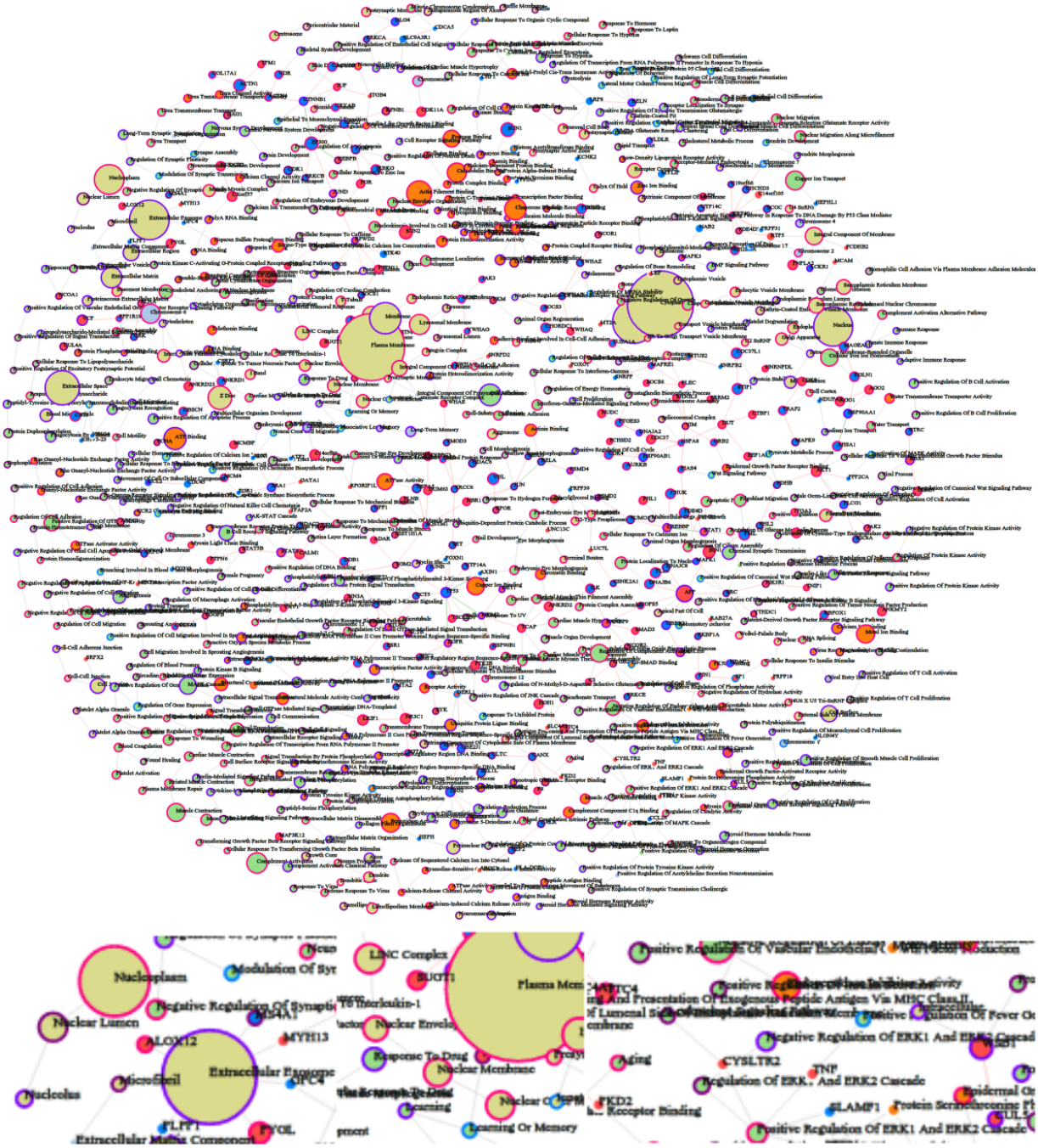

Figure 13: H. sapiens Ageing. *Network of genes differentially expressed with ageing in humans.*

3.3 Results

Nodes are colour coded in the following way. Upregulated genes are filled red and downregulated genes are blue. Processes are green, functions are orange, and locations are yellow. Halos indicate respective significant associations with the same colour code. Nodes to be associated with upregulated and differential-expressed genes get magenta coloured halos, those that are associated with downregulated and differential expressed genes obtain violet coloured halos and those that were found to be significant associated with upregulated as well as downregulated have halos coloured in purple. Physical interactions are pink, genetic interactions are green and other edges like, *is involved in*, *exhibits* and *is located in* are grey. Node size represents significance.

3.3.1 Human

3.3.1.1 Normal Ageing

Homo sapiens has been gene expression profiled at different ages. Individual molecular signatures (as defined by (Wuttke & de Magalhaes, 2011) [Molecular Profiling to Decipher Ageing] of human ageing were derived from the gene expression profiles. A strict q-value threshold of 5e-10 was utilized to generate reasonable adequate-sized graph for visualization [Figure 13 H. sapiens Ageing].

Human ageing-upregulated genes are associated with participating in the nuclear envelope organization, nuclear migration, exhibiting chromatin, actin filament and chaperone binding as well as located in lysosomal membrane and the aggresome (see discussion for explanation). Human ageing-upregulated genes are significantly interacting with *LTF*, *APP*, *AKT1*, *SUN1*, and *EP300*. Human ageing-downregulated genes are associated with participating in the MAPK cascade, exhibit transcriptional activator activity RNA polymerase II core promoter proximal region sequence-specific binding and being located in transcription factor complex. Human ageing-downregulated genes interact significantly with *APP*, *EP300*, *DIO2*, *TP53*, *MARCH6*, and *ELOC*. Human ageing-differentially expressed genes are associated with participating in signal transduction, positive regulation of transcription from RNA polymerase II promoter, positive/negative regulation of cell proliferation, positive regulation of cell migration as well as inflammatory response and being located in the extracellular exosome. Human ageing differentially expressed genes significantly interact with *APP* and *EP300*.

3.3.1.2 Accelerated Ageing

Several genetic syndromes confer a phenotype of accelerated ageing. Tissue samples from individuals with HGPS were transcriptional profiled in various studies. Here a consensus signature was derived from them representing accelerated ageing in humans and used to find significant associations with a relative strict threshold (q-value 5e-10) [Figure 14 H. sapiens HGPS].

Accelerated ageing-upregulated genes are associated with being involved in cell adhesion and regulation of Notch signalling pathway, exhibiting calcium ion binding and being located extracellular (extracellular matrix, extracellular matrix organization, proteinaceous extracellular matrix, and extracellular space). Accelerated ageing-downregulated genes are associated with Wnt signalling (WNT4, FZD6, positive regulation of Wnt signalling pathway, positive regulation of non-canonical Wnt signalling pathway, and Wnt-protein binding), cell cycle/proliferation (regulation of cell cycle process and positive regulation of cell proliferation), cellular response to hypoxia and vitamin D as well as proteostasis (proteolysis, cysteine-type endopeptidase activity, and regulation of autophagy). Accelerated ageing-differentially expressed genes are associated with positive regulation of extrinsic apoptotic signalling pathway via death domain receptors, somatic stem cell population maintenance and negative regulation of cell migration.

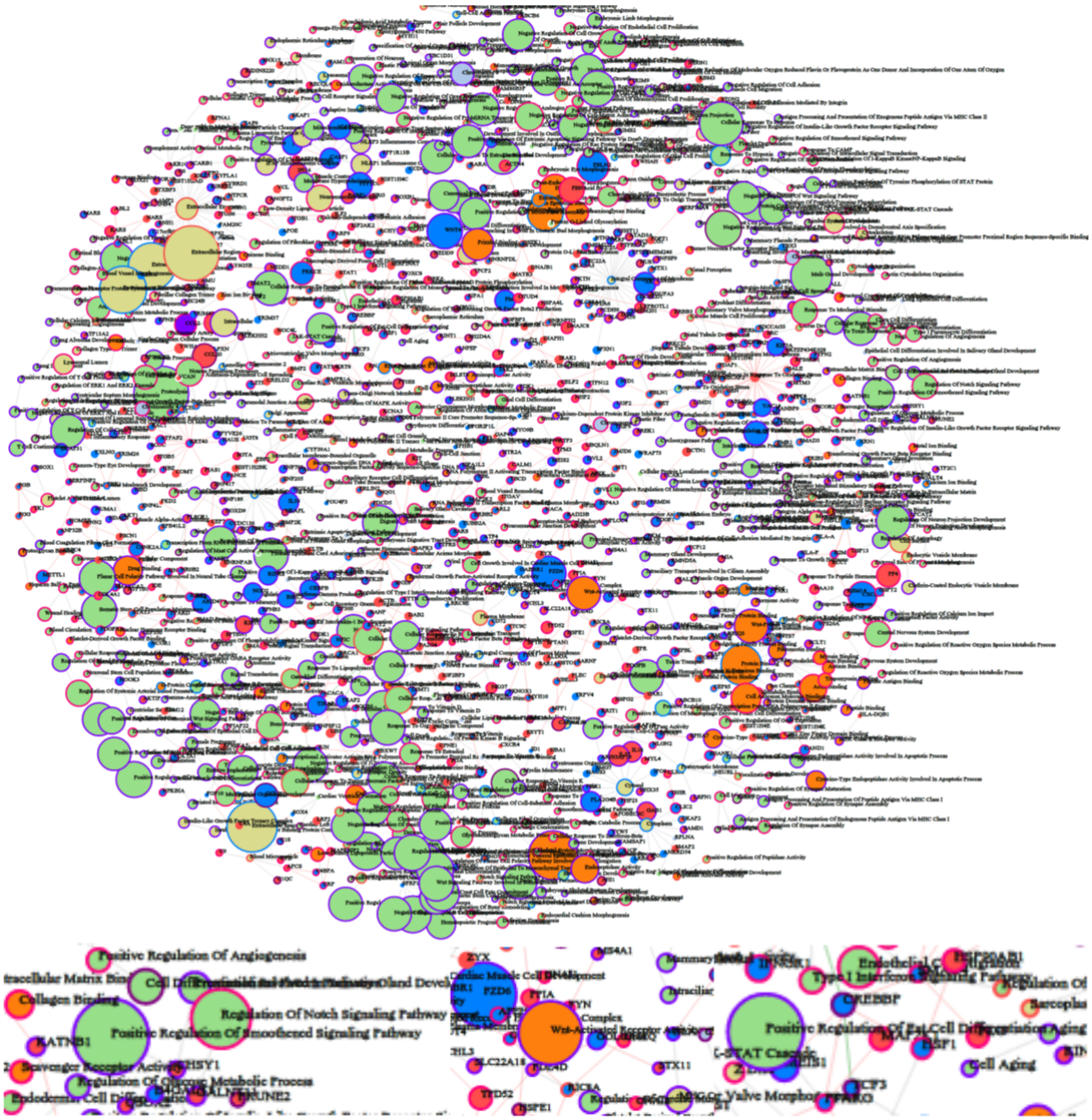

***Figure 14: H. sapiens HGPS**. Network of genes differentially expressed in accelerated ageing.*

3.3.1.3 Cellular Senescence

Cellular senescence can be induced by various mechanisms. Senescence cells have altered transcriptome in addition to other changed phenotypes that are characteristic for cellular senescence. A common consensus signature representing the transcriptional changes associated with cellular senescence by various ways have been established and subjected to the assessment of associations with a very string q-value threshold (5e-20) [Figure 15 H. sapiens Cellular Senescence].

Cellular senescence upregulated genes are associated with inflammation (inflammation, inflammatory response, cytokine activity, immune response, and positive regulation of NF-KappaB import into nucleus), extracellular space and cell-cell signalling, activation of MAPK activity, positive regulation of gene expression, positive regulation of transcription from RNA polymerase II promoter, negative regulation of cell proliferation, and extrinsic apoptotic signalling pathway in absence of ligand. Cellular senescence

downregulated genes are associated with cell cycle (G1S transition of mitotic cell cycle, G2M transition of mitotic cell cycle, DNA replication, and, cell division), ageing, wound healing, animal organ regeneration, extracellular matrix structural constituent, extracellular matrix organization, and being located in nucleus/nucleoplasm. Cellular senescence differentially expressed genes are associated with being involved in positive regulation of cell proliferation.

–

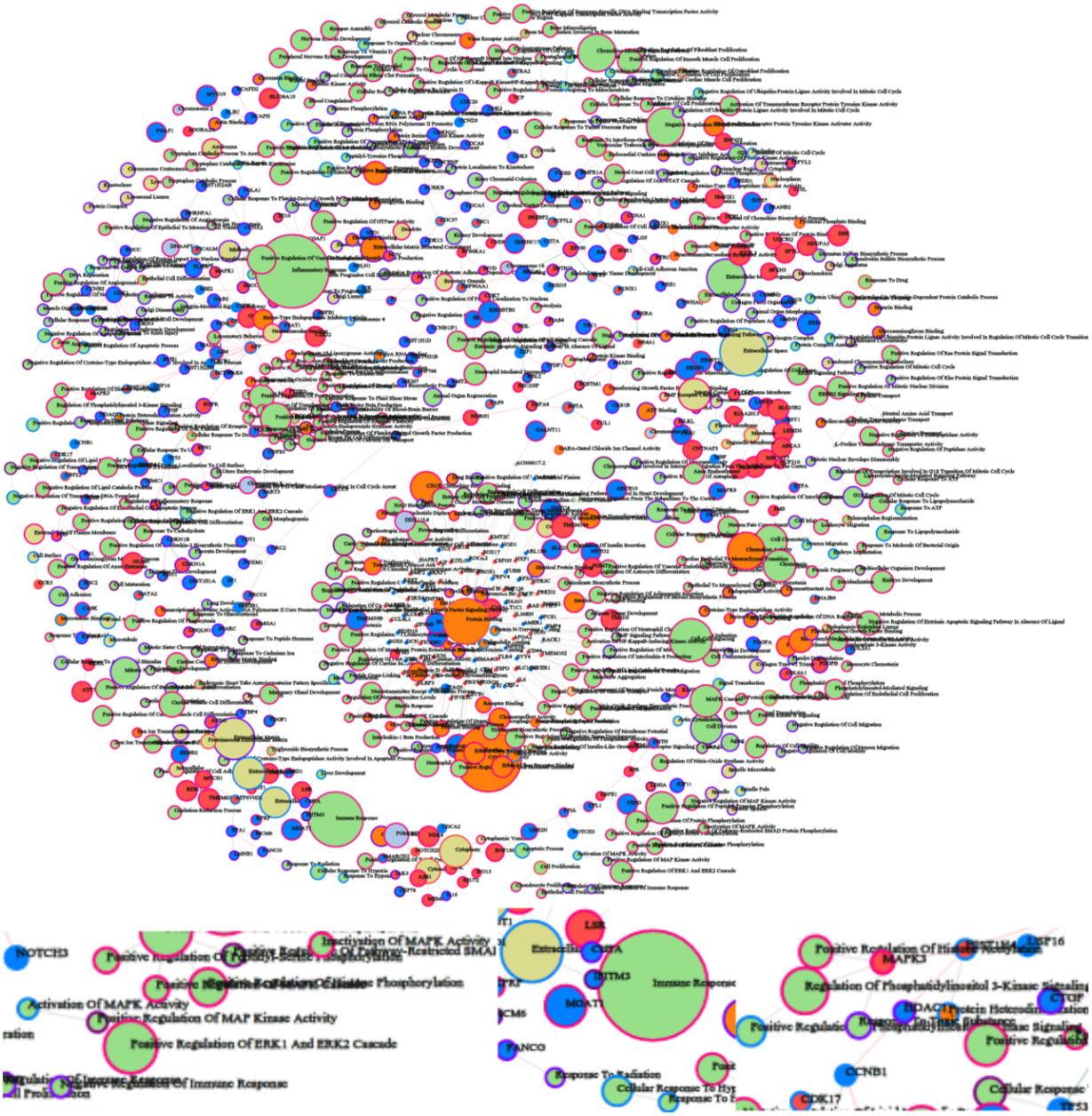

Figure 15: H. sapiens Cellular Senescence*. Network of genes differentially expressed in cellular senescence.*

3.3.2 Rat

Gene expression profiles of *Rattus norvegicus* with age annotations were utilized to derive a consensus signature of ageing. A q-value threshold of 5e-2 was used to visualize significant associations [Figure 16 R. norvegicus Ageing].

Rat ageing-upregulated genes are significantly associated with participating in immune response, inflammatory response, negative regulation of neurogenesis, exhibiting cytokine activity, being located in perinuclear region of cytoplasm, extracellular exosome, and being located on chromosome 13. Rat ageing-downregulated gene are significantly associated with response to nutrient levels, regulation of reactive oxygen species biosynthetic process, adaptive thermogenesis, cellular response to hormone stimulus, regulation of transcription from RNA polymerase II promoter and generation of precursor metabolites and energy, exhibiting oxidative phosphorylation uncoupler activity, are located on the mitochondrial envelope and are located on chromosome 19. Rat ageing-differentially expressed genes are significantly associated with ageing and interact with Ubc.

–

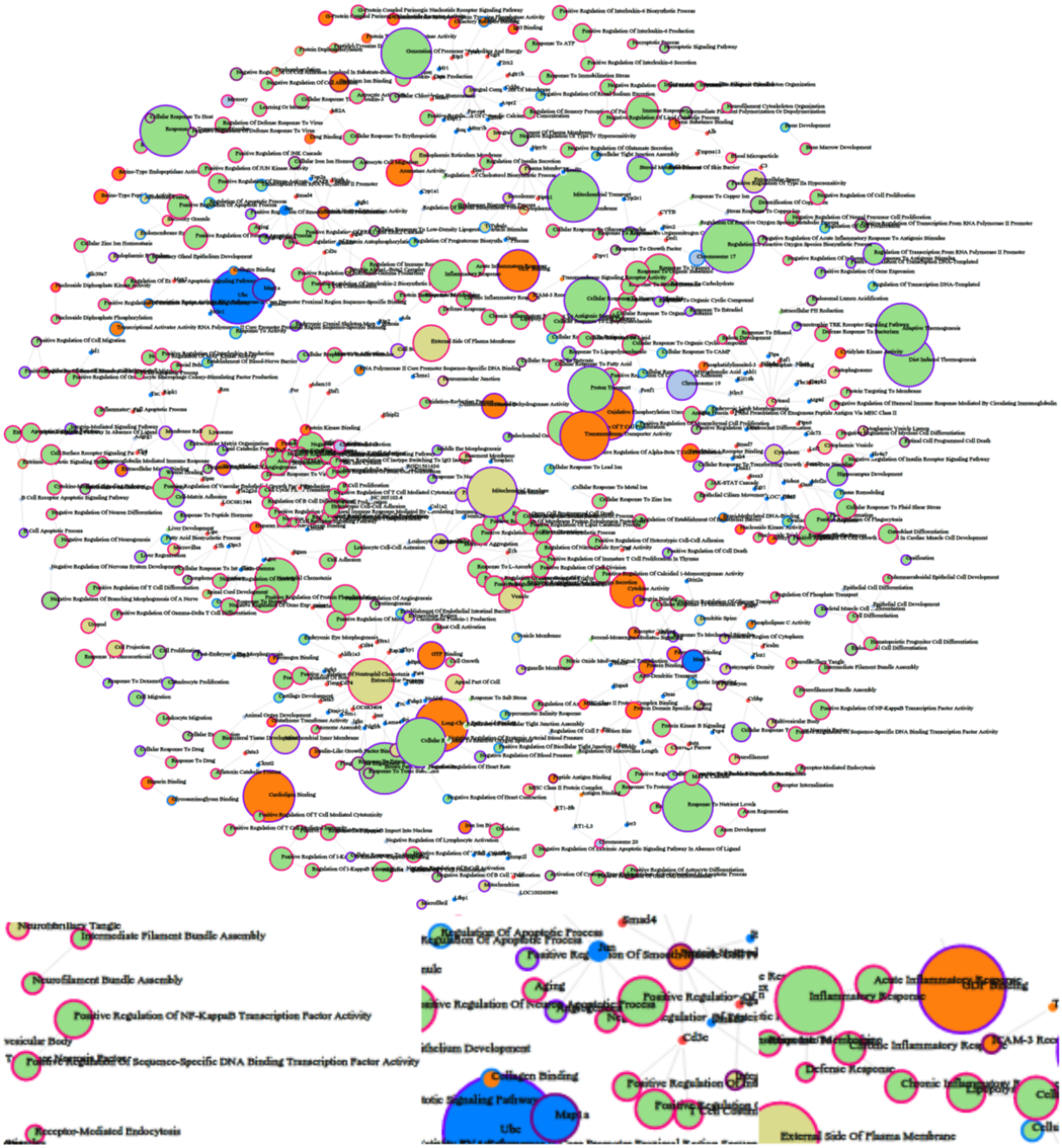

Figure 16: R. norvegicus Ageing. *Network of genes differentially expressed during ageing in rats.*

3.3.3 Mouse

–

***Figure 17: M. musculus Ageing**. Network of genes differentially expressed during ageing in mouse.*

Gene expression profiles in *M. musculus* were utilize to generate a murine consensus signature of ageing. Significant associations (q-value threshold of < 5e-5) are graphed [Figure 17 M. musculus Ageing].

Mouse ageing-upregulated genes are significantly associated with participating in cellular response to DNA damage stimulus, G2 DNA damage checkpoint, negative regulation of neuron projection development, and protein dephosphorylation, exhibiting beta-2-microglobulin binding and being located in extracellular exosome, nucleolus, and stress fiber. Mouse ageing-downregulated genes are significantly associated with participating in positive regulation of DNA-templated transcription elongation, mRNA processing, mRNA export from nucleus, regulation of proteasomal protein catabolic process, and protein deubiquitination, and exhibiting proteasome binding, and being located in proteasome complex and

transcription export complex. Mouse ageing differentially expressed genes are significantly associated with polyA RNA binding.

3.3.4 Fly

Drosophila melanogaster has been molecular profiled at different ages by several studies. Those profiles have been used for deriving a consensus signature of ageing in fly. The significant associations (q-value < 5e-2) are summarized as a graph [Figure 18 D. melongaster Ageing].

Fly ageing-upregulated genes are significantly associated with cellular response to heat, basement membrane disassembly, cell-cell junction organization, and tissue regeneration. Fly ageing-downregulated genes are significantly associated with participating in regulation of rho protein signal transduction and interacting with clu. Fly ageing-differentially expressed genes are significantly associated with participating in response to hyperoxia.

Figure 18: D. melongaster Ageing. *Network of genes differentially expressed during ageing in fruit fly.*

3.3.5 Worm

–

Figure 19: C. elegans Ageing*. Network of genes differentially expressed during ageing in nematode.*

Caenorhabditis elegans was profiled at different life stages. A consensus signature representing ageing of nematode has been established. Significant associations found within this signature have been graphed [Figure 19 C. elegans Ageing]. Worm ageing-upregulated genes are significantly associated with participating in protein dephosphorylation, nucleosome assembly, positive regulation of error-prone translesion synthesis, exhibiting hormone activity and unfolded protein binding, being located on

chromosome IV, and physically interacting with hsp-12.2. Worm ageing-downregulated genes are significantly associated with participating in lipid homeostasis, meiotic nuclear division, and proteolysis, exhibiting lysozyme activity and folic acid binding, being located on chromosome X, and physically interacting with act-1.

3.3.6 Yeast

–

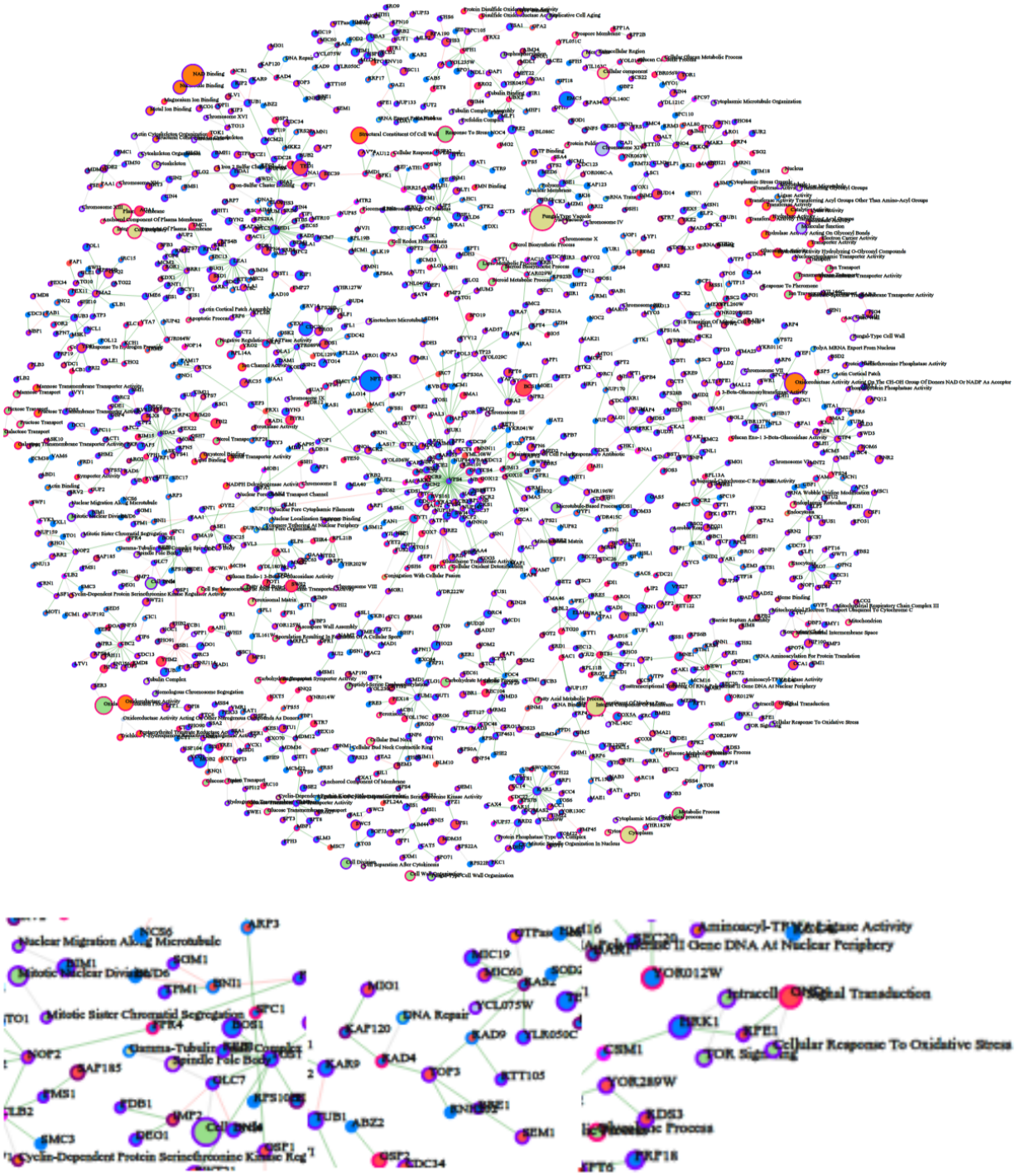

Figure 20: S. cerevisiae Ageing*. Network of genes differentially expressed during ageing in budding yeast.*

Saccharomyces cerevisiae gene expressions were profiled throughout its life in various studies. From this a consensus signature of ageing in budding yeast was created. Significant associations (q-value < 5e-5) have been found and illustrated in a graph [Figure 20 S. cerevisiae Ageing]. Yeast ageing-upregulated genes are significantly associated with oxidation-reduction process, response to stress, exhibiting transferase activity transferring acyl groups and being located in fungal-type vacuole and peroxisomal matrix. Yeast ageing-downregulated genes are significantly associated with participating in replicative cell ageing, cell cycle, G1S transition of mitotic cell cycle and actin cytoskeleton organization exhibiting oxidoreductase activity acting on the CH-OH group of donors NAD or NADP as acceptor, and interacting with ACT1.

–

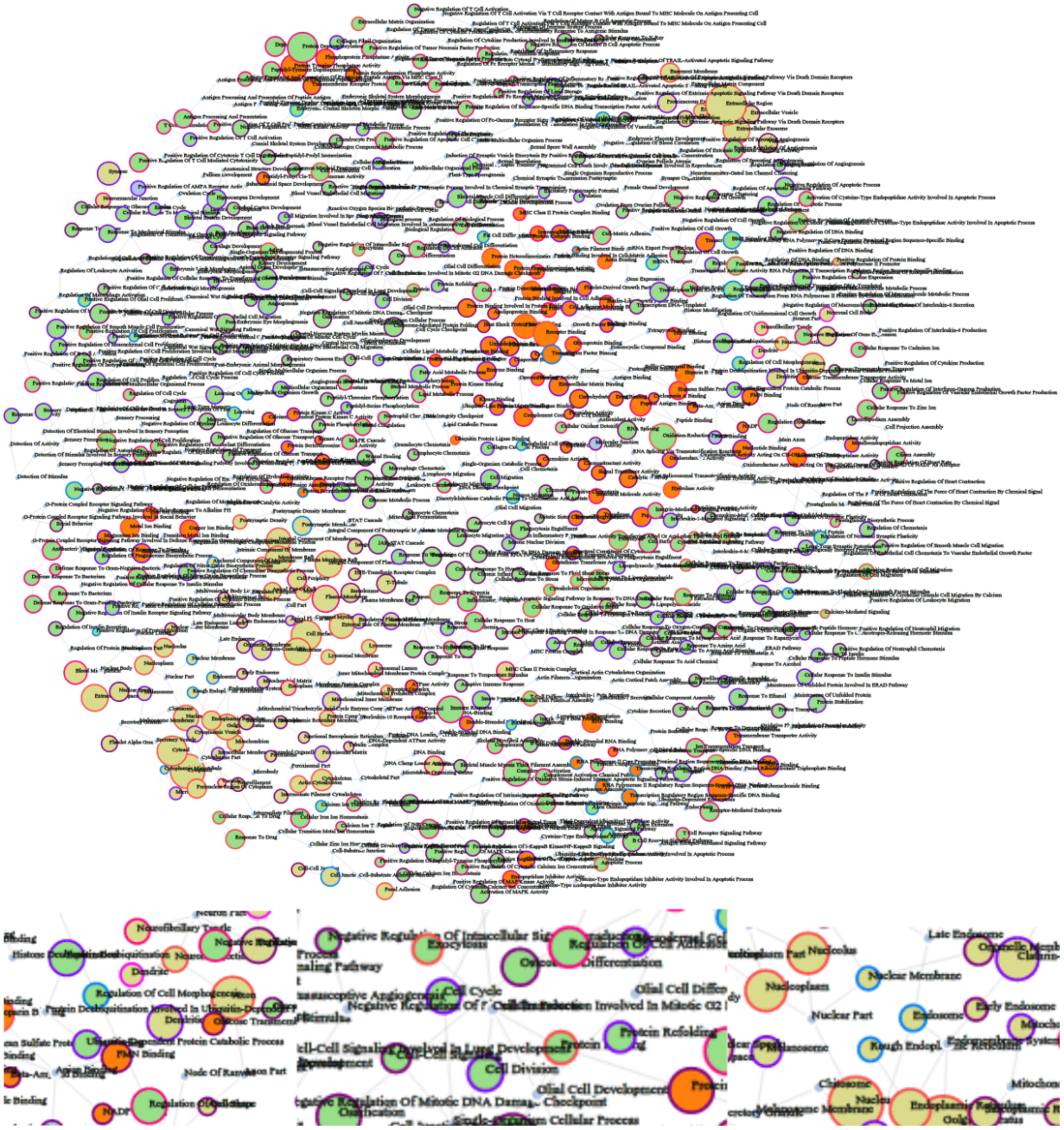

***Figure 21: Common Ageing**. Network of associations with ageing-differentially expressed genes commonly shared between multiple species.*

3.3.7 Common Ageing Signature Across Species

The consensus signatures of human rat, mouse, fly, worm and yeast have been subjected individually to Principal Angle Enrichment Analysis. The results for ageing-upregulated and ageing-downregulated were separately merged by count and combining q-values. Associations that are significant in more than half of the signatures and have a q-value of < 0.0005 are graphed [Figure 21 Common Ageing].

Genes upregulated with ageing are commonly significantly associated with endopeptidase (endopeptidase inhibitor activity, negative regulation of endopeptidase activity), inflammation (immune response, regulation of immune response, and positive regulation of NF-KappaB transcription factor activity), iron (iron ion transport, iron ion homeostasis), gene regulation (nucleosomal DNA binding, negative regulation of DNA binding, negative regulation of transcription DNA-templated, regulation of sequence-specific DNA binding transcription factor activity, positive regulation of sequence-specific DNA binding transcription factor activity, RNA polymerase II distal enhancer sequence-specific DNA binding, negative regulation of transcription from RNA polymerase II promoter, and RNA splicing), MAPK (MAPK cascade, activation of MAPK activity, positive regulation of ERK1 and ERK2 cascade), regulation of TOR signalling, regulation of cell shape, cellular response to heat, negative regulation of growth, and positive regulation of apoptotic processes.

Genes downregulated with ageing are commonly significantly associated with apoptosis (regulation of apoptotic process and negative regulation of neuron apoptotic process), insulin (response to insulin and insulin receptor binding), learning/memory (learning or memory, associative learning, and long-term memory), proteostasis (response to unfolded protein, chaperone binding, chaperone-mediated protein folding, chaperone mediated protein folding requiring cofactor, thiol-dependent ubiquitin-specific protease activity, protein transport, proteasome binding, recycling endosome), NAD binding, cell cycle, growth (multicellular organism growth), gene regulation (DNA binding, transcription DNA-templated, regulation of transcription DNA-templated, transcription regulatory region DNA binding, transcription regulatory region sequence-specific DNA binding, sequence-specific DNA binding, RNA polymerase II regulatory region sequence-specific DNA binding, and RNA polymerase II core promoter proximal region sequence-specific DNA binding), epigenetics (hemi-methylated DNA-binding, double-stranded methylated DNA binding, promoter-specific chromatin binding, and histone acetyltransferase binding), and Wnt (Wnt signalling pathway and negative regulation of canonical Wnt signalling pathway) as well as negative regulation of TOR signalling, and negative regulation of inflammatory response.

3.4 Discussion

Tissue as well as gender-specific signatures were combined to derive consensus molecular signatures of ageing for human and common biomedical model organisms and subsequently characterized for associations. A number of categories differentially expressed in the common consensus signatures of ageing can be interpreted with the current state of knowledge about the ageing process.

3.4.1 Genomic Instability

3.4.1.1 DNA Damage

Ageing-differentially expressed genes are commonly significantly associated with cellular response to DNA damage stimulus and intrinsic apoptotic signalling pathway in response to DNA damage by p53 class mediator [Common Ageing Signature Across Species]. More specifically ageing-upregulated genes are significantly associated with DNA damage response signal transduction by p53 class mediator resulting in transcription of p21 class mediator [Human]. p21 is upregulated during ageing with a positive characteristic direction [Human]. Genes downregulated with ageing are significantly associated with positive regulation of DNA repair [Normal Ageing; Yeast] and base-excision repair as well as nucleotide-excision repair DNA gap filling [Mouse]. This would mean that DNA repair is downregulated during ageing. Genes downregulated in cellular senescence are also involved in DNA repair [Cellular Senescence].

It was suggested that human ageing can be triggered by two main mechanisms which are telomere shortening and DNA damage. Telomere shortening and dysfunction may lead to DNA damage responses that induce cellular senescence. DNA damage accumulates, along with DNA repair deficiencies, resulting

in genomic instability and accelerated cellular senescence. Ageing due to telomere shortening or DNA damage is strongly dependent on p53. Both mechanisms can act cooperatively to increase the overall level of genomic instability and therefore trigger the onset of ageing phenotypes (Ding & Shen, 2008). Severe DNA damaged cells can either go into apoptosis or cellular senescence. However organisms have sophisticated repair systems that can cope with DNA damage.

3.4.1.2 DNA Repair

DNA repair is commonly significantly associated with ageing-differentially expressed genes [Common Ageing Signature Across Species]. Ageing-downregulated genes are significantly associated with positive regulation of DNA repair [Human], base-excision repair [Mouse], nucleotide-excision repair DNA gap filling [Mouse], DNA repair [Yeast], while ageing-upregulated genes are significantly associated with transcription-coupled nucleotide-excision repair [Mouse] and DNA damage stimulus [Figure 17 M. musculus Ageing].

DNA damages occur continuously in cells of organisms. Although most of these damages are repaired, some may accumulate. Especially in non-dividing cells DNA damage could accumulate. Accumulated DNA damages could interfere with RNA transcription in turn. Therefore the decline in the ability of DNA to serve as a template for gene expression was suspected to be the primary cause of ageing (Holmes, et al., 1992). Yet ageing-upregulated genes are also significantly associated with G2 DNA damage checkpoint that is a cell cycle checkpoint [Mouse] that detects and negatively regulates progression from G2 to M phase in the cell cycle in response to DNA damage. This could mean that cells are prevented from continuing in the cell cycle in favour of repairing damage.

The positive associations between age and levels of oxidative stress-generated damage to DNA is related to an age-dependent decline in DNA repair activity. There is an inverse association between age and DNA repair activity with a 0.65% decline in activity per year from 18 to 83 years in peripheral blood mononuclear cells of humans (Lohr, et al., 2015).

Ageing-upregulated genes are significantly associated with being involved in positive regulation of error-prone translesion synthesis [Figure 17 C. elegans Ageing]. Translesion DNA synthesis contributes to the accumulation of DNA mutations (Zou, et al., 2016).

3.4.1.3 Chromosomes

Ageing-downregulated genes are commonly significantly associated with chromosome organization and regulation of chromosome organization [Common Ageing Signature Across Species]. Ageing-upregulated genes are associated with condensed nuclear chromosome and negative regulation of chromosome condensation [Human]. Similar senescence downregulated genes are significantly associated with chromosome segregation, condensed chromosome, chromosome condensation and nuclear chromosome telomeric region [Cellular Senescence].

Organization of chromosomes has not been much investigated in relation to ageing. It is however clear that genomes are organized into complex higher-order structure by folding of the DNA into chromatin fibers, chromosome domains, and ultimately chromosomes. This higher-order organization of genomes is functionally important for gene regulation and control of gene expression programs. Changes in how chromatin is globally organized are relevant for physiological and pathological processes. Global alterations in chromatin structure are crucial for maintenance of genome stability, hence for ageing and tumourigenesis (Misteli, 2010).

Ageing, accelerated ageing, and cellular senescence all significantly upregulate genes located on chromosome 2 [Human]. Intriguingly, during development genes located on chromosome 2 are significant downregulated.

3.4.1.4 Telomere Attrition

Ageing-downregulated genes are commonly significantly associated with positive regulation of telomerase activity [Common Ageing Signature Across Species]. Moreover, ageing-downregulated genes are significantly associated with positive regulation of telomerase RNA localization to Cajal body (a class of nuclear body that may be involved in transcription) [Mouse], positive regulation of establishment of protein localization to telomere [Mouse], negative regulation of chromatin silencing at telomere [Yeast], and telomere tethering at nuclear periphery [Yeast]. Telomerase TERT itself is downregulated during ageing with a negative characteristics direction [Human]. Therefore, there is a downregulation of telomere maintenance with ageing. Telomerase activity is directly correlated with the expression of its active catalytic component (Ahmed & Tollefsbol, 2003). The disappearance of telomerase activity with ageing could be a natural defence against development of cancer (Ahmed & Tollefsbol, 2003) and/or a natural limitation of proliferation potential and lifespan.

Ageing-differentially expressed genes interact significantly with APP [Figure 13 H. sapiens Ageing]. APP encodes the amyloid beta (Abeta) precursor protein. Amyloid protein increases linear with age (30 - 89 years) (Rodrigue, et al., 2012). The aggregated form of Abeta, but not Abeta monomer, can inhibit telomerase activity both *in vitro* and in living cells (Wang, et al., 2015). APP transcript has a positive characteristic direction with ageing [Human]. Ageing and senescence differentially expressed genes (both up- and down-regulated) significantly interact with APP [Human]. Abeta oligomers inhibit telomerase activity through binding to DNA-RNA hybrid formed by telomeric DNA and the RNA template of telomerase, then blocking telomerase elongation of telomeric DNA. Intracellular Abeta localizes at telomere, and induces cellular senescence and telomere shortening (Wang, et al., 2015).

3.4.2 Epigenetic Alterations

3.4.2.1 Nuclear Organization

The nuclear envelope organization and nuclear migration have been found to be significantly associated with ageing-upregulated genes [Figure 13 H. sapiens Ageing]. Nuclear organization and nuclear envelope proteins have been suspected for playing a role in ageing (Ye & Bhalla, 2011).

Ageing-upregulated genes are significantly associated with lamin binding [Human]. The nuclear lamina is a meshwork of lamins and lamina-associated proteins, which provide mechanical support, control size and shape of the nucleus, as well as mediate the attachment of chromatin to the nuclear envelope. Abnormal nuclear shapes are observed in ageing cells. The size of the nuclei increases with age and nuclei assume an aberrant shape (Brandt, et al., 2008).

3.4.2.2 Chromatin

Ageing-upregulated genes are significantly associated with negative regulation of chromatin silencing [Human], while chromatin silencing is itself significantly associated with ageing-downregulated genes [Mouse]. Ageing-downregulated genes are significantly associated with chromatin organization [Common Ageing Signature Across Species], nuclear chromatin [Mouse], (nuclear) heterochromatin [Mouse], nuclear euchromatin [Rat] chromatin silencing [Mouse], promoter-specific chromatin binding [Human, Rat], and negative regulation of chromatin silencing at telomere [Yeast]. Ageing-upregulated genes are significantly associated with chromatin binding [Human], telomeric heterochromatin assembly [Human], ATP-dependent chromatin remodelling [Mouse], and covalent chromatin modification [Rat]. Ageing-differentially expressed genes are significantly associated with senescence-associated heterochromatin focus [Mouse] and pericentric heterochromatin assembly [Human].

There is a age-related loss of repressive heterochromatin which is associated with a loss of gene silencing in yeast and metazoans (Jiang, et al., 2013). The complex macromolecular structure of chromatin regulates all nuclear processes requiring access to the DNA primary sequence. Maintenance of chromatin structure is an integral component to deter premature ageing. Histone modification impact on chromatin compaction and gene expression and undergo numerous changes during ageing. Lifespan can be robustly extended by interventions that reverse age-dependent changes in chromatin structure. This all indicates to a pivotal role of chromatin structure plays during ageing (Feser & Tyler, 2011).

The differential expression of genes involved in senescence-associated heterochromatin during normal ageing could be due to that fact that aged cells are more primed to convert into the senescence state, or a higher proportion of the cells of aged organisms have become already senescence.

Ageing-differentially expressed and upregulated genes are significantly associated with the NuRD complex [Mouse]. However the crucial components of this complex are actually downregulated [Human, Mouse]. NuRD (Nucleosome Remodelling Deacetylase) complex is a group of associated proteins with both ATP-dependent chromatin remodelling and histone deacetylase activities (Xue, et al., 1998; Zhang & Yinghua, 2010). HDAC1 is significantly associated with downregulated genes in ageing [Human, Mouse], premature ageing [Human] and senescence [Human]. HDAC1 and HDAC2 are both downregulated as they have negative characteristic directions [Human, Mouse]. Physiological and premature ageing are characterized by multiple changes in chromatin structure and accumulation of persistent DNA damage. NURD chromatin remodelling complex is a key modulator of these ageing-associated chromatin changes. There is a loss of multiple NURD components during premature and normal ageing including an ageing-associated reduction in HDAC1 activity. Silencing individual NURD subunits recapitulates chromatin changes associated with ageing. The structural chromatin changes precede DNA damage accumulation (Pegoraro, et al., 2009).

3.4.2.3 DNA Methylation

Maintenance of DNA methylation is significantly associated with ageing-upregulated genes [Human], while DNA methylation itself is significantly associated with both ageing-upregulated [Mouse] and ageing-downregulated genes [Rat]. Hemi-methylated DNA binding and double-stranded methylated DNA binding is significantly associated with ageing-downregulated genes [Human; Rat].

DNA methylation is usually associated with epigenetic silencing of genomic loci. Rather than being a stable modification, DNA methylation and demethylation are dynamic (Kouzmenko, et al., 2010). DNA methylation increases throughout lifespan (Haghighi, et al., 2014).

3.4.2.4 Histones

Ageing-differentially expressed genes and ageing-downregulated genes are commonly and significantly associated with histone acetyltransferase binding [Common Ageing Signature Across Species]. Ageing-upregulated genes are significantly associated with histone binding [Human], lysine-acetylated histone binding [Human], histone deacetylase binding, histone H3 deacetylation [Mouse] and methylated histone binding [Mouse]. Ageing-downregulated genes are significantly associated with histone acetyltransferase binding [Common Ageing Signature Across Species; Human; Rat], positive regulation of histone acetylation [Rat], histone H3-T6 phosphorylation [Human], histone kinase activity H3-T6 specific [Human], histone demethylase activity [Human], histone demethylase activity H3-K27 specific [Human], histone H3-K27 demethylation [Human], histone deacetylase binding [Human], and positive regulation of histone deacetylase activity [Human]. H3K9 acetylation significantly decreases with age (Kawakami, et al., 2009). H3K27 is associated with transcriptional repression (Ma, et al., 2015).

Senescence upregulated genes are significantly associated with positive regulation of histone phosphorylation and acetylation, while senescence downregulated genes are significantly associated with histone phosphorylation and histone kinase activity [Cellular Senescence]. Senescent cells display nuclear foci of phosphorylated histone H2AX (d Adda di Fagagna, et al., 2003). MSK1 (mitogen- and stress-activated kinase) mediates histone H3S28 phosphorylation at the INK4AB/ARF locus and contributes to the rapid transcriptional activation of p15INK4b and p16INK4a (cellular senescent marker) in human cells despite the presence of the repressive H3K27me3 mark (Culerrier, et al., 2016).

3.4.3 Transcriptional Regulation

Ageing-upregulated genes are commonly significantly associated with positive regulation of NF-KappaB transcription factor activity [Common Ageing Signature Across Species]. Constitutive activation of NF-KappaB is a ubiquitous phenomenon among various cell types in the ageing phenotype (Kriete & Mayo, 2009).

Ageing-differentially expressed genes interact significantly with EP300 [Figure 13 H. sapiens Ageing]. EP300 is a transcriptional coactivator with histone acetyltransferase activity. EP300 activity attenuates with age in several tissues in mice (Li, et al., 2002). Its nematode ortholog *cbp-1* is an ageing-suppressor gene and essential for the lifespan extension mediated by dietary restriction.

TP53, the guardian of the genome, is significantly associated with interacting with both up- and down-regulated genes during ageing and senescence. TP53 is a ortholog of a ageing gene [Figure 13 H. sapiens Ageing].

Transcription factors like EGR1 were found to be commonly downregulated [Figure 13 H. sapiens Ageing] and could be responsible to orchestrates age-related gene expression changes. Age-related reduction in the transcription of Egr1 is due to CpG site-specific change in the methylation in DNA associated with the promoter region of Egr1 (Penner, et al., 2016).

3.4.4 Post-Transcriptional Processing

During ageing there is a decoupling of transcript and protein levels conserved between primates to yeast. Ageing-downregulated genes are associated with transcription (Wei, et al., 2015).

mRNA processing and mRNA export from nucleus, polyA RNA binding and transcription export complex are significantly associated with ageing-downregulated genes [Figure 17 M. musculus Ageing]. This downregulation might lead to the observed discrepancy in transcript and protein levels.

3.4.5 Proteostasis

Ageing-downregulated genes are significantly associated with exhibiting unfolded protein binding and being involved in response to unfolded protein [Figure 19 Common Ageing]. Young organisms pose an efficient response to unfolded proteins but older organisms exhibit an impairment in unfolded protein response to stresses (Naidoo, et al., 2008).

3.4.5.1 Chaperoning

Ageing-upregulated genes have been found to be significantly associated with exhibiting chaperone binding [Figure 13 H. sapiens Ageing]. Chaperones have a crucial role in the repair of proteotoxic damage, which is greatly increased in aged subjects. Upregulation of genes exhibiting chaperone binding during ageing could block their availability and reduce their ability to repair proteotoxic damage. Hence the observed increase in proteotoxicity with ageing (Nardai, et al., 2002).

With ageing there is decreased chaperone capacity. The decrease in chaperone capacity may reflect a direct proteotoxic damage of chaperones or an increase in chaperone occupancy resulting in a "chaperone overload" due to the increased amount of damaged proteins in aged cells (Nardai, et al., 2002).

3.4.5.2 Aggresome

Ageing-upregulated genes are significantly associated with being located in the aggresome [Figure 13 H. sapiens Ageing]. The aggresome is an inclusion body formed by dynein-dependent retrograde transport of aggregated protein on microtubules. Decreased proteolysis is caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and "aggresomes" during oxidative stress, ageing and degenerative diseases (Grune, et al., 2004).

Protein aggregation is a common feature of several neurodegenerative diseases and to a certain extend of normal ageing. Not always is it clear, why protein aggregation takes place, however a disturbance in the balance between protein synthesis and protein degradation appears to be important. Resulting from this is the accumulation of modified proteins, which have a tendency to form high molecular weight aggregates. Those aggregates are also called inclusion bodies, plaques, lipofuscin, ceroid, or "aggresome" depending on their location and what they are composed of. These aggregates are not inert metabolic end products, but rather actively influence cell metabolism, especially proteasomal activity and protein turnover (Grune, et al., 2004).

3.4.5.3 Advanced Glycation Endproducts

RAGE receptor binding is upregulated during ageing [Rat]. This means genes encoding proteins that interact selectively and non-covalently with the RAGE receptor are upregulated.

RAGE is the receptor for advanced glycation endproducts (AGE) which are increasing with age (Haus, et al., 2007). AGEs include glycoproteins, i.e. glycans that have been modified non-enzymatically through the Maillard reaction and are assumed to be side products of metabolism but also considered to have signalling functions (Ott, et al., 2014). AGEs increase Sirt1 ubiquitination and proteasome-mediated degradation, hence shorten Sirt1 half-life, and subsequently promote fibronectin (FN) and TGF-beta1 expression (Huang, et al., 2015).

3.4.5.4 Ubiquitin-Proteasome System

The ubiquitin-proteasome system is associated with ageing-downregulated genes with regulation of proteasomal protein catabolic process, and protein deubiquitination, proteasome binding, as well as proteasome complex [Figure 17 M. musculus Ageing]. The interplay between molecular chaperones, ubiquitin/deubiquitinating enzymes and proteasomes is critical in protein homeostasis especially during both ageing and heat stress (Oling, et al., 2014).

Ageing-upregulated genes are significantly participating in negative regulation of endopeptidase activity [Figure 11 H. sapiens Ageing] and with this could reduce the extend of proteolysis. Proteasome inhibition is known to occur during normal ageing and contributes towards age-related increase in oxidative stress (Ding, et al., 2006).

Commonly to ageing across species is the upregulation of genes that are significantly associated with exhibiting endopeptidase inhibitor activity [Figure 21 Common Ageing]. Endopeptidase inhibitor activity stops, prevents or reduces the activity of endopeptidases (any enzyme that hydrolysis non-terminal peptide bonds in polypeptides) and therefore potential inhibits proteolysis of proteins. Similar protein transport and proteolysis are commonly downregulated.

Thiol-dependent ubiquitin-specific protease activity is associated commonly with ageing-downregulated genes [Figure 19 Common Ageing]. This activity catalysis the thiol-dependent hydrolysis of a peptide bond formed by the C-terminal glycine of ubiquitin and another protein. USP22 is significantly associated with ageing-downregulated genes. USP22 itself is downregulated during ageing [Normal Ageing] and in senescence [Cellular Senescence]. USP22 depletion enhances Sirt1 degradation and displays a combined effects with AGEs to further promote FN and TGF-beta1 expression (Huang, et al., 2015).

3.4.5.5 Lysosome

Ageing-upregulated genes are significantly associated with being located at the lysosomal membrane [Figure 11 H. sapiens Ageing; Figure 18 S. cerevisiae Ageing]. A wide pool of cytosolic proteins are selectively degraded in lysosomes, especially by chaperone-mediated autophagy (CMA). Binding of proteins involved in CMA to a receptor at the lysosomal membrane is a rate limiting step in CMA. If the receptors at the lysosomal membrane are obstructed due to other proteins are being located at the lysosomal membrane this could block the process of CMA and possible other lysosomal activities that mediate repair of damaged cellular components (Kaushik, et al., 2007).

3.4.5.6 Autophagy

Negative regulation of autophagy is associated with ageing differentially expressed genes [Figure 13 H. sapiens Ageing; Figure 19 C. elegans Ageing; Figure 21 Common Ageing]. The function of autophagy declines with increasing age (Cavallini, et al., 2008).

Accelerated ageing downregulates proteostasis including proteolysis and autophagy regulation [Figure 14 H. sapiens HGPS]. Proteasome activity and autophagy are impaired in HGPS cells (Gabriel, et al., 2015).

3.4.6 Cytoskeleton

Ageing-downregulated genes are commonly significantly associated with structural constituent of cytoskeleton and negative regulation of cytoskeleton organization [Common Ageing Signature Across Species]. Increased organization of cytoskeleton accompanies ageing. There is an increased organization of microfilaments into bundles in senescence cells. The age-related increase in cell size is correlated with increased organisation of the cytoskeleton (Wang & Gundersen, 1984).

3.4.6.1 Actin

Actin was found to be significantly associated with ageing differentially expressed genes in multiple organisms from human to yeast [Figure 13 H. sapiens Ageing; Figure 19 C. elegans Ageing; Figure 20 S. cerevisiae Ageing]. Actin is associated with cell movement, cell division and intracellular transport.

Mutants with altered actin dynamics exhibit increased lifespan (Tocchetti, et al., 2010). Alleles that decrease actin dynamics increase chronological lifespan, while alleles that increase actin dynamics also enhance chronological lifespan (Gourlay, et al., 2004).

3.4.6.2 Microtubule

Ageing-upregulated genes are commonly significantly associated with microtubule anchoring and microtubule cytoskeleton organization [Common Ageing Signature Across Species]. Ageing-downregulated genes are commonly significantly associated with microtubule, microtubule cytoskeleton, and cytoplasmic microtubule [Common Ageing Signature Across Species].

Senescence cells exhibit an increase in the abundance of microtubules per cell and the distribution pattern is altered through the increase in the number of organization centres (Wang & Gundersen, 1984).

The regulation of cell shape is significantly associated commonly with ageing-upregulated genes [Figure 19 Common Ageing]. Microtubules are ubiquitous cellular components that are involved in the control of cell structure and function, like cell division, regulation of shape and polarity as well as intracellular transport among others. Ageing of cells is characterized by the appearance of various cell dysfunctions (Raes, 1991).

3.4.7 Inflammation

Ageing-differentially expressed genes and especially ageing-upregulated genes are significantly associated with participating in inflammatory response immune response and exhibiting cytokine activity [Figure 13 H. sapiens Ageing; Figure 16 R. norvegicus Ageing; Common Ageing Signature Across Species]. Genes upregulated during accelerated ageing are also significantly associated with regulation of inflammatory response [Accelerated Ageing]. Inflammation increases with age, hence the existence of the concept of "inflammageing" (Giunta, 2008).

Ageing-upregulated genes are commonly associated with positive regulation of inflammatory response, while ageing-downregulated genes are associated with negative regulation of inflammatory response [Figure 21 Common Ageing]. Thus, ageing overall is associated with enhancement of inflammation.

Cellular senescence upregulates inflammation, negative regulation of cell proliferation and apoptosis, while downregulates cell cycle, wound healing and regeneration [Cellular Senescence]. Senescence cells irreversible cease cell cycling (Takahashi, et al., 2007), become resistance to apoptosis (Wang, 1995; Schauble, et al., 2012; Ryu, et al., 2007) and exhibit a distinct inflammatory phenotype (Kuilman, et al., 2010; Rodier & Campisi, 2011). Cellular senescence fuels inflammation associated ageing and cancer progress (Rodier & Campisi, 2011). Inflammatory responses trigger and transmit senescence to neighbouring cells and activate senescence-associated secretory phenotype (Long, et al., 2016).

3.4.8 Signalling

3.4.8.1 MAPK

The mitogen-activated protein kinase cascade (MAPK) plays a regulatory role in signal transduction. Ageing-differentially expressed genes are commonly significantly involved in MAPK cascade, activation of MAPK activity, inactivation of MAPK activity, activation of MAPKK activity, and positive regulation of MAPK cascade [Common Ageing Signature Across Species]. Specifically ageing-upregulated genes are commonly significantly associated with MAPK cascade and activation of MAPK activity [Common Ageing Signature Across Species].

It was suggested that the age-related decline in antioxidant defence is closely involved with the expression of Nrf2 (alias NFE2L2/Nfe2l2) and is regulated by the MAPK family (Shih & Yen, 2007). NFE2L2/Nfe2l2 exhibits negative characteristic direction (i.e. is downregulated) in the human and mouse consensus signature. NFE2L2 is a orthologous to several ageing genes (Tang & Choe, 2015).

3.4.8.2 TOR

Ageing-differentially expressed genes and in particular ageing-upregulated genes are commonly significantly associated with regulation of TOR signalling [Common Ageing Signature Across Species]. Ageing-downregulated genes are commonly significantly associated with negative regulation of TOR signalling [Common Ageing Signature Across Species].

AKT1 is significantly associated with ageing-upregulated genes. AKT1 is an ortholog of ageing genes [Figure 13 H. sapiens Ageing]. AKT1 was found to be associated with longevity (Pawlikowska, et al., 2009; Nygaard, et al., 2013).

3.4.8.3 Wnt

Wnt signalling pathway is significantly associated with premature ageing-downregulated genes [Accelerated Ageing] but also commonly with normal ageing-downregulated genes [Common Ageing Signature Across Species].

More specifically, genes downregulated in premature ageing are associated with positive regulation of canonical Wnt signalling pathway, non-canonical Wnt signalling pathway, Wnt signalling pathway involved in somitogenesis, Wnt-protein binding, Wnt-activated receptor activity, WNT4, positive regulation of Wnt signalling pathway, and positive regulation of canonical Wnt signalling pathway [Accelerated Ageing].

3.4.8.4 Notch

Premature ageing-upregulated genes are significantly associated with being involved in Notch signalling pathway, regulation of Notch signalling pathway, and Notch signalling involved in heart development, as well as interacting with NOTCH1 [Accelerated Ageing].

Negative regulation of Notch signalling pathway is significantly associated with ageing-downregulated genes [Rat]. Cellular senescence upregulated genes significantly interact with NOTCH2NL [Cellular Senescence].

3.4.9 Regeneration

3.4.9.1 Stem Cells

Ageing-downregulated genes are significantly associated with somatic stem cell division, male germ-line stem cell asymmetric division, stem cell factor receptor binding, somatic stem cell population maintenance, hematopoietic stem cell migration [Common Ageing Signature Across Species].

Ageing-upregulated genes are significantly associated with negative regulation of neurogenesis [Figure 16 R. norvegicus Ageing]. Neurogenesis in mammals occurs throughout life (and is assumed to be important for the laying down of the episodic memory) diminishes significantly with increasing age (Gray, et al., 2002). Similar ageing-upregulated genes are significantly associated with participating in negative

regulation of neuron projection development which is any process that decreases the rate, frequency or extent of neuron projection development [Figure 17 M. musculus Ageing].

Accelerated ageing differentially expresses somatic stem cell population [Accelerated Ageing], upregulates Notch signalling [Accelerated Ageing] and strongly downregulates Wnt signalling [Accelerated Ageing; Figure 14 H. sapiens HGPS]. Mutations that cause HGPS affect adult stem cells by interfering with Notch (induction) and Wnt (suppression) signalling pathway (Meshorer & Gruenbaum, 2008).

3.4.9.2 Wound Healing

Wound healing and response to wound healing are commonly significantly associated with ageing-differentially expressed genes. More specifically, negative regulation of response to wounding is commonly significantly associated with ageing-upregulated genes [Common Ageing Signature Across Species].

Advanced age is commonly a risk factor for delayed wound healing. Impaired wound healing is due to inherent differences in cellular structure and function that impair tissue repair and regeneration. Cellular senescence results in slowed or impaired wound healing in the elderly (Thomas, 2001). Impaired wound healing in aged individuals presents a major clinical and economic problem (Gosain & DiPietro, 2004).

3.4.10 Ions

Ageing-upregulated genes participate in copper ion binding and iron transport [Figure 13 H. sapiens Ageing] that might be related to the availability of metal ions in the cell. Metal ions are used as cofactors by enzymes but also lead to the enhanced generation of free radicals (Gutteridge, 1985).

Accelerated ageing upregulates calcium ion binding [Figure 14 H. sapiens HGPS]. Proteins associated with calcium ion binding were also found to be upregulated in HGPS. More strikingly free cytosolic calcium is increased in HGPS (Harten, et al., 2011).

3.4.11 Extracellular

Ageing-upregulated genes significantly participate in extracellular matrix organization [Figure 13 H. sapiens Ageing]. Ageing is accompanied by changes in the composition and structure of the extracellular matrix, resulting in changes in the mechanics of connective tissues in older individuals. These progressive dysfunctions facilitate numerous human pathologies and deficits associated with ageing, including cardiovascular, musculoskeletal and neurodegenerative disorders and diseases (Phillip, et al., 2015).

Accelerated ageing upregulates extracellular entities [Figure 14 H. sapiens HGPS]. The extracellular matrix dysregulation observed in HGPS is implicated as a factor in disease progression. In HGPS there is a shift in the inherent extracellular matrix-degrading proteolytic balance in favour of matrix deposition (Harten, et al., 2011).

Ageing-upregulated genes are significantly located in the extracellular exosome [Figure 13 H. sapiens Ageing; Figure 16 R. norvegicus Ageing; Figure 17 M. musculus Ageing]. Extracellular exosomes (exosomes for short) are extracellular vesicles secreted by cells that carry cargo from cells like proteins and RNAs. Exosomes are used by senescence cells as part of the senescence-associated secretory phenotype to convey senescence signals (Urbanelli, et al., 2016). Exosomal miRNAs are very predictive biomarkers of Alzheimer's disease (Lugli, et al., 2015). Salivary exosomal miRNAs are useful ageing biomarkers as well (Machida, et al., 2015).

3.4.12 Integrins

Cell to extracellular matrix interactions are mediated by integrins. Integrins are adhesion molecules that regulate a number of processes and developmental events.

Ageing-upregulated genes significantly participate in integrin-mediated signalling [Figure 13 H. sapiens Ageing]. Integrin signalling mutants life longer, indicating that functional senescence and age-dependent mortality are influenced by integrin signalling in *C. elegans* (Hansen, et al., 2005) and *D. melanogaster* (Goddeeris, et al., 2003; Nishimura, et al., 2014).

3.4.13 Hormones

Ageing-downregulated genes are significantly associated with being involved in cellular response to hormone stimulus [Figure 16 R. norvegicus Ageing]. There is hormone imbalance with increasing age and aged cells tend to become insensitive to certain hormonal triggers. Ageing-downregulated genes are significantly associated with being involved in response to nutrient levels [Figure 16 R. norvegicus Ageing]. Older animals might not respond so well to dietary restriction.

Accelerated ageing downregulates the response to hypoxia and vitamin D [Figure 14 H. sapiens HGPS]. HGPS cells reduce expression of vitamin D receptor (VDR). Reconstituting VDR signalling via 1alpha,25-dihydroxyvitamin D3 (1,25D) treatment improves HGPS phenotypes, including nuclear morphological abnormalities, DNA repair defects, and premature senescence (Kreienkamp, et al., 2016). Moreover HGPS cells have severe increased hypoxia sensitivity. It is hypothesized that this decreased stress resistance causes additional depletion of the mesenchymal stem cell pool responsible for replacing differentiated cells lost to progerin toxicity (Zhang, et al., 2011).

3.4.14 Mitochondrial Dysfunction

3.4.14.1 Mitochondrion

Both ageing-upregulated and downregulated genes are commonly significantly associated with mitochondrion. Genes downregulated with ageing are much more commonly associated with mitochondria. More specifically, ageing-downregulated genes are commonly significantly associated with mitochondrial part, mitochondrial inner membrane, mitochondrial matrix, establishment of protein localization to mitochondrion, protein localization to mitochondrion, mitochondrial transport, and mitochondrial transmembrane transport [Common Ageing Signature Across Species]. All this downregulation could lead to the well known phenomenon of mitochondrial dysfunction that accompanies ageing.

3.4.14.2 Energy Metabolism

Ageing-downregulated genes are significantly associated with being involved in generation of precursor metabolites and energy [Figure 16 R. norvegicus Ageing]. This downregulation might further result into mitochondrial dysfunction. Most of those enzymes are contained in the mitochondria. Mitochondria are dynamic organelles critical for many cellular processes, including apoptosis but also energy generation. Indeed changes in the expression of proteins important for biological processes involved in the generation of precursor metabolites and energy through the breakdown of carbohydrates, lipids and proteins were observed (Stauch, et al., 2015).

Similar ageing-downregulated genes are significantly associated with exhibiting oxidative phosphorylation uncoupler activity [Figure 16 R. norvegicus Ageing]. Mitochondrial inefficiency through proton leak, uncouples substrate oxidation from ADP phosphorylation and compromise as much as 30% of the basal metabolic rate. Such uncoupling is assumed to protect cells from conditions that favour ROS production, but also generates heat. Uncoupling can also be achieved through pharmaceutical induction of proton leak and activity of a class of uncoupler proteins. Pharmaceutics that uncouple or overexpression mitochondrial uncoupler proteins can extend the lifespan of model organisms (Mookerjee, et al., 2010; Brand, et al., 2004). Therefore, a downregulation of uncoupler might be associated with negative effects. Further ageing-downregulated genes are significantly associated with being located in the mitochondrial envelope. The mitochondrial envelope is the double lipid bilayer enclosing the mitochondrion and separate its contents from the cell cytoplasm (includes the intermembrane space). Downregulation of genes that encode products located in this compartment could induce even more mitochondrial dysfunctions (Kalous & Drahota, 1996; Unknown, 2008; Bratic & Larsson, 2013).

3.4.14.3 Reactive Oxygen Species

Ageing-upregulated genes are commonly significantly associated with response to reactive oxygen species [Common Ageing Signature Across Species]. Ageing-downregulated genes are significantly associated with being involved in reactive oxygen species metabolic process and regulation of reactive oxygen species biosynthetic process [Figure 16 R. norvegicus Ageing]. More specifically ageing-downregulated genes are significantly associated with negative regulation of reactive oxygen species biosynthetic process [Common Ageing Signature Across Species]. Similar accelerated ageing-upregulated genes are significantly involved in regulation of reactive oxygen species metabolic process and in particular positive regulation of reactive oxygen species metabolic process [Accelerated Ageing].

This could lead to increased synthesis of reactive oxygen species biosynthesis in both normal ageing and premature ageing. The accumulation of molecular damage from the attack by reactive oxygen species was once thought to be one cause of ageing (de Castro, et al., 2004).

3.4.14.4 Thermogenesis

Ageing-downregulated genes are significantly associated with being involved in adaptive thermogenesis [Figure 16 R. norvegicus Ageing]. Also diet-induced thermogenesis is associated significantly with ageing-downregulated genes [Figure 16 R. norvegicus Ageing].

The ability to regulate body temperature diminishes with age (Prat & Roberge, 1960; Scarpace, et al., 1994). The capacity for diet-induced thermogenesis declines with age and is virtually absent in old organisms (Rothwell & Stock, 1983).

3.4.14.5 NAD

Ageing-downregulated genes are commonly significantly associated with exhibiting NAD binding [Common Ageing Signature Across Species]. Also oxidoreductase activity acting on the CH-OH group of donors NAD or NADP as acceptor is significantly associated with ageing-downregulated genes [Figure 20 S. cerevisiae Ageing]. The NAD to NADH ratio was found to impact on lifespan in a variety of model organisms. The intracellular ratio of pyridine nucleotides has been proposed to be at the centre stage of age-related biochemical changes in organisms and may also help to explain the influence of dietary restriction and energy-sensing proteins in lifespan (de Carbo, et al., 2009).

NAMPT was found to be commonly downregulated (low negative characteristic direction) in multiple tissues and might therefore reduce overall tissue NAD levels [Normal Ageing]. NAMPT catalyses the rate-limiting step in NAD biosynthesis that is critical in energy metabolism, cell senescence and maintaining lifespan. NAMPT also influences stem cell senescence. Nampt expression is significantly lower in aged mesenchymal stem cells. Pharmacological inhibition of Nampt young mesenchymal stem cells are induced to become aged cells. Nampt overexpression attenuates cell senescence in aged mesenchymal stem cells (Ma, et al., 2017).

3.4.15 Lipid Homeostasis

Ageing-downregulated genes are significantly associated with participating in lipid homeostasis [Figure 19 C. elegans Ageing]. Ageing is accompanied by pronounced deposition of lipids in non-adipose tissues (including the nervous system). Interventions that promote longevity such as low insulin signalling, germline loss and dietary restriction, effectively delay ageing in evolutionary divergent organisms, and diminish the rate of ectopic fat accumulation (Palikaras, et al., 2016).

Ageing-downregulated genes are commonly significantly associated with lipid catabolic process [Common Ageing Signature Across Species]. Ageing-upregulated genes are commonly significantly associated with positive regulation of lipid storage [Common Ageing Signature Across Species]

Ageing-upregulated genes are significantly associated with plasma membrane repair [Common Ageing Signature Across Species]. Plasma membrane repair is the resealing of a cell plasma membrane after cellular wounding due to for instance, mechanical stress. This might mean that older cells are more exposed to stress that disrupts the plasma membrane.

3.4.16 Apoptosis

Ageing upregulated and downregulated genes are commonly significantly involved in apoptotic process and activation of cysteine-type endopeptidase activity involved in apoptotic process. Ageing-downregulated genes are associated with apoptotic process whereas ageing-upregulated genes are specifically associated with positive regulation of apoptotic processes [Figure 21 Common Ageing. Ageing-upregulated genes are commonly significantly involved in positive regulation of apoptotic process and positive regulation of apoptotic cell clearance. Ageing-downregulated genes are commonly significantly involved in regulation of apoptotic process, negative regulation of apoptotic process, and negative regulation of neuron apoptotic process [Common Ageing Signature Across Species].

Accelerated ageing differentially regulates apoptosis [Figure 14 H. sapiens HGPS]. Proteins associated with apoptosis were found to be differentially expressed as well in HGPS (Wang, et al., 2012).

Apoptosis plays both positive and negative roles in ageing. Of particular importance is the role in disrupting tissue homeostasis and promoting neurodegenerative disease (Warner, 1999). RAGE activation increases reactive oxygen species production which in turn mediates apoptosis (Chuang, et al., 2007).

3.4.17 Memory

Ageing-downregulated genes are commonly associated with memory, long-term memory, and learning or memory [Figure 21 Common Ageing]. In addition, short-term memory is significantly associated with ageing-downregulated genes [Normal Ageing].

Aged organisms experience a reduction in both short-term and long-term memory (Zornetzer, et al., 1982). There are also age-related impairments in reward-based learning (Eppinger, et al., 2010). Older adults typically perform more poorly than do younger adults on many types of memory tasks. The reduction in the speed of information processing is a fundamental contributor to normal age-related memory loss (Luszcz & Bryan, 1999).

3.4.18 Stress Response

Common to ageing-upregulated genes there is a significant association of involvement in the cellular response to heat [Figure 18 D. melongaster Ageing; Figure 21 Common Ageing]. This might be the upregulation of negative regulators overall of heat stress response or a compensating response of cells to the increased proteotoxicity.

In organisms, intracellular and extracellular proteins are constantly subjected to a variety of spontaneous non-enzymatic modifications that overall affect their structure and function, as well as stability. For instance, protein oxidation and glycation are tightly linked and are implicated in the development of numerous pathological consequences of ageing. Although multiple endogenous pathways in the cell can prevent the formation of modified (oxidized or glycated) proteins, and repair or degrade the abnormal proteins, abnormal proteins do accumulate with ageing. The heat-shock response (involving the family of heat shock proteins) represents the quickest and most conserved response to proteotoxic insults (Verbeke, et al., 2000).

3.4.19 Peroxisome

Ageing-differentially expressed genes are commonly and significantly associated with peroxisome, peroxisomal part (compartment, i.e. any constituent part of a peroxisome) and peroxisomal matrix. Specifically ageing-downregulated genes are commonly significantly associated with the peroxisomal matrix [Common Ageing Signature Across Species]. Further peroxisome proliferator-activated receptor binding is significantly associated with ageing-downregulated genes.

The peroxisome is a small organelle enclosed by a single membrane, and found in most eukaryotic cells. Peroxisomes contain peroxidases and other enzymes involved in a variety of metabolic processes including importantly free radical detoxification as well as lipid catabolism and biosynthesis, and hydrogen peroxide metabolism. Disturbance in the functions of peroxisome also contributes to ageing (Williams, 2014).

Peroxisome proliferator-activated receptor is a group of nuclear receptor proteins that function as transcription factors. PPARA and PPARD are downregulated with ageing but significantly interact with ageing-upregulated genes [Human]. PPARA polymorphism is associated with ageing (Naito, et al., 2007).

3.4.20 Lifespan/Ageing Controllers

Ageing downregulated genes are significantly associated with being involved in determination of adult lifespan [Worm] and replicative cell ageing [Figure 20 S. cerevisiae Ageing]. Downregulation of ageing-suppressor genes could be an effective mechanism of the ageing process.

Accelerated ageing differential and downregulated genes are significantly associated with cell ageing [Accelerated Ageing]. Cellular senescence also differentially expresses genes involved in cell ageing [Cellular Senescence].

3.4.21 Growth Regulation

Cell growth is associated with ageing-upregulated genes, while multicellular organism growth, regulation of cell growth, positive regulation of cell growth are all associated with ageing-downregulated genes. This is in line with the hypothesis that the developmental growth cessation program is still active during ageing and leads to loss of functions [Figure 21 Common Ageing].

Downregulated genes are also associated with cellular component organization [Common Ageing Signature Across Species]. Proper organization of cellular components are necessary for life. Disorganization of cellular components could be a hallmark of ageing.

3.4.22 Cell Differentiation & Proliferation/Cycle

Ageing-upregulated genes are associated with regulation of differentiation and proliferation. In particular ageing-upregulated genes are commonly associated with cell differentiation and negative regulation of cell proliferation [Figure 21 Common Ageing]. The various tissues could have in common to become highly differentiated and lose proliferation potential. Positive regulation of cell death is also enriched for association with ageing-upregulated genes with is in line with that the loss of cells in tissues give raise to organ dysfunction and various age-related diseases, like diabetes and neurodegenerations.

Moreover, cell division and positive regulation of cell cycle is commonly significantly associated with ageing-downregulated genes, while negative regulation of G1S transition of mitotic cell cycle is commonly significantly associated with ageing-upregulated genes [Common Ageing Signature Across Species]. This regulation indicates that the cell cycle is negatively regulated and expected to decline with increasing age. Downregulation of cell cycle with increasing age, might make some type of cancer less severe with increasing age, but at the same time reduces regenerative capacity and leads to loss of cells.

Ageing-downregulated genes are significantly associated with participating G1S transition of mitotic cell cycle [Figure 20 S. cerevisiae Ageing]. Ageing cells have a prolonged cell cycle (Austriaco, 1996).

p21 (*CDKN1A*) is highly upregulated gene (high positive characteristic direction) during ageing common to multiple tissues and may prevent regeneration and wound healing [Normal Ageing]. p21 is also upregulated in accelerated ageing and cellular senescence [Accelerated Ageing; Cellular Senescence].

Furthermore, S100 protein binding was found to be upregulated [Human]. S100 proteins are localized both in the cytoplasm as well as in the nucleus in a broad spectrum of different cells. They are involved in regulation of a number of cellular process like cell cycle progression and differentiation. During ageing tissues become more and more differentiated and cell cycling reduces progressively for certain types of cells, while it increases in abnormal cells like cancer cells. The class of S100 genes include at least 13 members of which most are located as a cluster on chromosome 1q21. In adults S100 proteins usually have elevated expression due to nervous system damage, which makes them a potential clinical marker.

Previous attempt of deriving a transcriptional consensus signature of ageing identified upregulation of immune response, lysosome, extracellular region of plasma membrane, glycoprotein signal and negative regulation of apoptosis, and downregulation of mitochondrion, oxidative phosphorylation, cytoplasm, and

hydroxylysine/hydroxylation/collagen (de Magalhaes, et al., 2009). Immune response and in particular inflammation was found here to be commonly associated with ageing-upregulated genes and specifically the positive regulation of inflammatory activities. Ageing-downregulated genes, on the contrary, are commonly associated with negative regulation of inflammation. Additionally cellular senescence was also found to upregulate inflammation and also HGPS upregulates the regulation of inflammation [Inflammation]. Genes upregulated as well as downregulated with ageing have both been found here to be commonly significantly associated with mitochondrion. However there are many more significant associations of ageing-downregulated genes with mitochondria related processes, activities and locations, indicative of increased mitochondrial dysfunction with ageing [Mitochondrial Dysfunction].

3.5 Conclusion

Here molecular signatures reflecting ageing in humans and model organisms were derived, primarily by using transcriptomics. Tissue-specific and common consensus signatures were generated. This technique could be extended to other omics if sufficient data is available (e.g. epigenomics, proteomics, metabolomics, etc.). Differentially methylated regions and genes could be identified and common signatures of ageing methylation changes be derived. Similarly, differentially expressed proteins could be obtained from the proteomics data and common signatures of protein level changes generated. Furthermore, metabolomics could be used to create common signatures of metabolite levels from differential expressed metabolites with ageing.

Transcriptional consensus signatures of ageing revealed that ageing is accompanied by upregulation of inflammation, differentiation and suppression of proliferation, growth retardation, increased apoptosis, and downregulation of proteostasis.

4 Dietary Restriction Genes

Abstract: Dietary restriction (DR), limiting nutrient intake from diet without causing malnutrition, delays the ageing process and extends lifespan in multiple organisms. The conserved life-extending effect of DR suggests the involvement of fundamental mechanisms, although these remain a subject of debate. To help decipher the life-extending mechanisms of DR, a list of genes that if genetically altered disrupt or prevent the life-extending effects of DR was compiled. These genes were called DR-essential genes and more than 100 were identified in model organisms such as yeast, worms, flies, and mice. In order for other researchers to benefit from this first curated list of genes essential for DR, an online database called GenDR (http://genomics.senescence.info/diet/) was established. To dissect the interactions of DR-essential genes and discover the underlying lifespan-extending mechanisms, a variety of network and systems biology approaches were used to analyse the gene network of DR. It is here shown that DR-essential genes are more conserved at the molecular level and have more molecular interactions than expected by chance. Furthermore, a guilt-by-association method to predict novel DR-essential genes was employed. In budding yeast, nine genes related to vacuolar functions were predicated; Here it is experimentally shown that mutations deleting eight of those genes prevent the life-extending effects of DR. Three of these mutants (*OPT2*, *FRE6*, and *RCR2*) had extended lifespan under *ad libitum*, indicating that the lack of further longevity under DR is not caused by a general compromise of fitness. These results demonstrate how network analyses of DR using GenDR can be used to make phenotypically relevant predictions. Moreover, gene-regulatory circuits reveal that the DR-induced transcriptional signature in yeast involves nutrient-sensing, stress responses and meiotic transcription factors. Finally, comparing the influence of gene expression changes during DR on the interactomes of multiple organisms suggests that DR commonly suppresses translation, while stimulating an ancient reproduction-related process.

4.1 Background

Dietary restriction (DR) in defined factors (such as calories or specific amino acids) without causing malnutrition delays the ageing process, protects against age-related diseases (e.g. metabolic and cardiovascular disease, neurodegeneration and cancer) and extends lifespan in evolutionarily distant species, from unicellular yeast to rodents (Bishop & Guarente, 2007; Fontana, et al., 2010). Furthermore, there is evidence that DR delays the ageing process in non-human primates (Kemnitz, 2011) and perhaps in humans as well (Willcox, et al., 2007a). Caloric restriction in the presence of adequate nutrition is effective in delaying the effects of ageing. Older age of onset, female sex, lower body weight and fat mass, reduced food intake, diet quality, and lower fasting blood glucose levels are factors associated with fewer age-related disorders and with improved survival. Reduced food intake was specifically beneficial in adult and older primates, but not in younger monkeys (Mattison, et al., 2017; Kumlien, 2017).

While multiple studies in model organisms have shown that DR extends lifespan, the underlying molecular mechanisms remain largely unknown (Fontana, et al., 2010; Spindler, 2010; de Magalhaes, et al., 2012).

The mechanisms by which DR retards ageing have been shown in model organisms to be mediated by genetic pathways, many of which are evolutionarily conserved and operate in humans. Considerable evidence indicates that DR is mediated by discrete signalling events that elicit specific genetic programs (Bishop & Guarente, 2007; Fontana, et al., 2010; Lakowski & Hekimi, 1998; Partridge, et al., 2005). Mutations in a number of genes, in fact, have been shown to prevent, shift or disrupt the lifespan-extending effect of DR, in many cases without altering the lifespan under *ad libitum* (AL) conditions. Such genes, which are designated as DR-essential, might specifically interfere with these signalling cascades and the program that leads to lifespan extension under DR. Understanding the interactions of such genes in a systematic way may provide important new clues regarding DR-mediated life-extension mechanisms.

In the post-genomic era, the vast amount of omics data provides an opportunity to understand biological processes in a systematic fashion via data integration, network construction and analyses. Networks in biology exist on various levels, derived from the interactions between genes, transcripts, proteins and metabolites, and extending to the interactions between cells, tissues, organs and even between organisms. Examples at the molecular level are protein-protein interactions, gene-regulatory and metabolic networks. Biological networks tend to have certain discrete topological properties: They are usually scale-free (i.e. only a few nodes, termed “hubs”, have many connections, while the majority of nodes have relatively few connections), modularly composed and hierarchically structured. Scale-free networks are robust against random perturbation, but at the same time are sensitive to targeted disruption of the hubs (Barabasi, et al., 2011). The modular composition is due to the presence of groups of highly interconnected nodes (i.e. clusters) which perform certain biological activities (for instance protein complexes or pathways) with relatively sparse external connectivity. Lastly, these network modules associate with one another in a hierarchical manner. The challenge, however, is to integrate and combine the various networks in order to decipher biological function and gain new insights into the process under study.

Ageing-associated genes are more conserved at the molecular level (de Magalhaes & Church, 2007) and have a significantly higher node degree (i.e. number of interactions in a network) than expected by chance. Human orthologs of ageing-associated genes found in model organisms form a continuous network and almost all of the hubs are also implicated in several age-related diseases (Budovsky, et al., 2007). The networks of age-related disease genes and ageing-associated genes significantly overlap and approximately half of the common genes participate in signal transduction. Additionally, general disease genes have more connections to ageing genes than expected by chance (Wang, et al., 2009). Numerous network studies on ageing have been conducted with fruitful results (Promislow, 2004; Witten & Bonchev, 2007; Fortney, et al., 2010; Bell, et al., 2009; Budovsky, et al., 2007; de Magalhaes & Toussaint, 2004; Li, et al., 2010; Lorenz, et al., 2009; Wang, et al., 2009). To date, however, network studies on DR have been limited, in part because of a lack of adequate datasets. Therefore, GenDR was constructed, a database of DR-related genes.

Dietary restriction has been shown to extend lifespan in diverse, evolutionarily distant species, yet its underlying mechanisms remain unknown. Firstly, a database of genes essential for the life-extending effects of dietary restriction in various model organisms was constructed and then studied for their interactions using a variety of network and systems biology approaches. This enabled to predict novel genes related to dietary restriction, that were validated experimentally in yeast. By comparing large-scale data compilations (interactomes and transcriptomes) from multiple organisms, it was possible to condense this omics information to the most conserved essential elements, eliminating species-specific adaptive responses. These results leads to the rather surprising conclusion that lifespan extension by a restricted diet commonly may exploit an ancient rejuvenation process derived from gametogenesis.

Since GenDR is the first database of DR-related genes, its utility was assessed by performing the first network-based dissection of DR using DR-essential genes. DR can induce numerous changes in organisms, and a variety of different DR regimens have been employed. The rationale is that by concentrating only on genes essential for DR-induced life-extension it is possible to narrow down the various DR-related processes to those affecting ageing. The list of DR-essential genes was used to investigate their evolutionary and network properties and identify common regulators of DR-induced lifespan extension. Novel DR-essential genes were predicted from the network and tested experimentally in yeast, which revealed new genes crucial for DR. Then diverse types of data were integrated and a variety of network and systems biology analyses performed to reveal common pathways and mediators of DR effects, including putative transcription factors. This work demonstrates the use of network approaches to study DR mechanisms, and makes list of DR-essential genes available online for other researchers to use (http://genomics.senescence.info/diet/).

4.2 Methods

4.2.1 Database Creation

A list of genes, termed DR-essential genes, which if genetically manipulated (knockout by deletion, or transposition, knockdown by RNA interference, or overexpression of transgenes) interfere with the ability of DR to extend lifespan in model organisms (budding and fission yeast, nematode, fruit fly, and mouse) was compiled from the literature. The focus on genes from genetic manipulations experiments means that the selection procedure for selecting genes related to DR will be more objective and unbiased. Genes were included if they interfere with at least one kind of DR regimen which also includes by definition a shift in the response to food concentration at which lifespan is extended (e.g. chico gene). A database (GenDR) was implemented in the relational database management system MySQL and a webpage interface was designed (http://genomics.senescence.info/diet/), as for other ageing-related databases (de Magalhaes, et al., 2005). Each entry in the database contains manually curated comments about literature-based evidence and the reason for inferring an association with DR. If there are conflicting reports for a given gene, the policy is to still include the gene in the database but mention all the conflicting reports and then let visitors make their own mind on how to interpret them. This neutral stance policy is similar to the one already employed for the GenAge database of ageing-related genes. Literature citations and links to PubMed are also given. Further, conserved gene expression changes upon DR in mammals are included, although these have been described in another work (Plank, et al., 2012).

4.2.2 Molecular Evolution

4.2.2.1 Assembling Orthologs

Homologs of genes in the GenDR database were retrieved from the NCBI HomoloGene Database, Ensembl/BioMart, OrthoMCL (Fischer, et al., 2011), and InParanoid (Ostlund, et al., 2010) by merging. Genes are either homologous or not, it is a binary assignment, therefore the union was taken by merging them. For the molecular evolution part specifically only BioMart (Ensembl Genes 57) was used to retrieve tables of orthologs (homologs between two different species) for *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, *Rattus norvegicus*, *Macaca mulatta* and *Homo sapiens*. Information about *Saccharomyces pombe* is not available in Ensembl and this species was therefore excluded from the following analyses. For calculating the presence of orthologs, Ensembl protein identifiers were employed to filter for only protein-coding genes. For a given set of genes, here DR-essential genes, it was counted how many of these genes in one species have also at least one protein-coding homologous gene in all the other species under study. This was also done for all protein-coding genes in each genome and given these two values the percentage of presence of homologs were estimated. All pairwise comparisons for each seed species were averaged and the Poisson p-values calculated.

4.2.2.2 Calculating Average dN, dS, and dN/dS Ratios

Non-synonymous (dN) and synonymous (dS) nucleotide substitution rates (de Magalhaes & Church, 2007; Koonin & Wolf, 2010) were retrieved from the Ensembl Compara built 65 for the orthologous pairs between a rodent (*M. musculus*), a non-human primate (*M. mulatta*) and humans (*H. sapiens*). Orthologs relationships were restricted to the top hits of InParanoid. In this sense, the number of paralogs is corrected for which might be a source of bias as big gene families tend to have higher dN/dS ratios. The average values for dN and dS as well as dN/dS ratios for the orthologous relationships of DR-essential genes were compared against those without orthologs of DR-essential genes. In order to avoid biases due to overrepresentation of ancient genes, only genes which had also orthologs in lower model organisms (*S. cerevisiae*, *C. elegans*, or *D. melanogaster*) were selected. Thus, the comparison was between ancient genes that are presumably essential for DR versus ancient genes with no known essential role in DR. The corresponding p-values for differences in dN, dS, and dN/dS were calculated with a Mann Whitney U test.

Invertebrates were not studied because of the difficulty to obtain dN and dS values.

4.2.3 Molecular Interaction Information

4.2.3.1 Integration

Interactions datasets were retrieved from IntAct (Download 23.01.2011; http://www.ebi.ac.uk/intact/; Aranda, et al., 2010), DIP (Update 10.10.2010) (Xenarios, et al., 2002), MINT (Version 2010-12-15 updated on 21/12/2010 11:07:00) (Ceol, et al., 2010), BIND (http://baderlab.org/BINDTranslation; Isserlin, et al., 2011), BioGRID (Release 3.1.72) (Breitkreutz, et al., 2008), MPACT (Guldener, et al., 2006), DroID (Version 2010_08), Reactome (Haw & Stein, 2012; Croft, et al., 2011; Stein, 2004), HPRD (Version 9) (Keshava Prasad, et al., 2009), PDZBase (Beuming, et al., 2005), CORUM (Ruepp, et al., 2010), iRefIndex (05182010) (Razick, et al., 2008), PhosphoSitePlus (Fri Dec 10 13:43:52 EST 2010) (Hornbeck, et al., 2004), PhosphoGRID (Stark, et al., 2010), I2D (Version 1.8) (Brown & Jurisica, 2007), InteroPORC (Michaut, et al., 2008), InterologFinder (Wiles, et al., 2010), MiMI (Jayapandian, et al., 2007), (Tarcea, et al., 2009), PINA (Update March 4, 2010) (Wu, et al., 2009b), YeastNet (Lee, et al., 2007), WormNet (Lee, et al., 2010), MouseNet (Kim, et al., 2008), and TF-Atlas (Ravasi, et al., 2010) preferentially as PSI-MAT or simple tab-delimited flat file format. Interactions were aggregated by using a unified schema consisting of synonyms for each gene/protein, their taxonomy identifiers, interaction type and detection system, PubMed identifiers for references, as well as source databases. For simplification, interactions between molecules other than genes, transcripts, or proteins were omitted and only interactions between genes/transcripts/proteins of the same species were considered.

4.2.3.2 Mapping and Merging

Symbols, names, aliases and various identifiers of genes, transcripts and proteins were in the first instance mapped to unique Entrez Gene IDs, Ensembl Gene IDs, UniProt IDs and species-specific IDs (from WormBase, MGI, etc.). Ensembl Gene, UniProt, or species-specific database IDs were if possible converted into Entrez Gene IDs. In a second iteration collected synonyms from Ensembl, UniProt, and species-specific database of genes/proteins with missing Entrez Gene IDs were used as queries to map them to synonymous tables of Entrez. In each mapping the identifier used had always the highest level of matches. For instance, Entrez Gene IDs were chosen with the highest consensus of all other database-derived synonymous tables.

The mapped interactions were merged to a directional merged interactome if the source and target interactors are the same as well as to an undirectional merged interactome in which all interactions describing the same interacting entities are fused regardless of source or target interactors.

For the integrated, mapped and merged interactions a suitable scoring system was developed. Interactions gain scores for each experimental system type, each different interaction detection method that was used, and additionally for each different publication which observed an interaction. Furthermore, if an interaction involves a post-translational modification event it receives a further score.

4.2.4 Network Analyses

The integrated interaction data was transferred into a relational database, queried with gene lists and networks visualized via custom Python scripts or with the use of Cytoscape (Cline, et al., 2007).

Each gene (candidate) was assessed for the number of interactions (degree) with genes in the query lists (seeds), as well as their total interaction number. As a measure of specificity, the ratio of specific interaction number with the seeds divided by the total number of interactions for each gene (seeds and candidates) was calculcated, as a percentage, and its corresponding p-value (based on the binomial distribution) determined. Genes with a binomial p-value < 0.05 were classified as significant interactors. Whether a set of genes has a higher average degree than expected by chance was determined using a Mann Whitney U test. An exhaustively leave-one-out test as applied by repeatedly omitting single genes from the seed list and tested whether the genes were among the significant candidate genes inferred from the remainder. From this procedure the percentage of retrieval was estimated for each of the seed genes by successively omitting each gene, one at a time, from the seed list.

4.2.5 Guilt-by-Association

Interaction information was retrieved from BioGRID (version Version 3.4.140), annotations on processes, functions, locations, and pathways were retrieved from the NCBI FTP server. The significance to be associated with DR-essential genes was calculated with the hypergeometric test. Significant associations plus the DR-essential gene seed list was graphed via automatic force-directed graph layout (Kobourov, 2012).

4.2.6 Correlation of Variables and Multiple Linear Regression

A multiple linear regression analysis was performed in Python focused on human genes that considers three variables: the degree of each gene in the interaction network, the number of species with orthologs, and the mean of its dN/dS ratios with three other mammalian species (*M. musculus*, *R. norvegicus* and *M. mulatta*). Genes with a degree of zero or with no given dN/dS ratios were removed, in order to log transform the data. A Pearson correlation was applied to first check whether any of those variables are correlated with each other. Then a regression analysis was conducted, by the method of least squares, of each variable against other variables first singly and then for a given variable as a function of the other two. The derived linear equations were used to predict the variables of greatest relevance for the set of DR-essential genes.

4.2.7 Functional Enrichment Analyses

By a False Discovery Rate (FDR) of <5% gene lists were examined using the DAVID bioinformatics resource (http://niaid.abcc.ncifcrf.gov/), under default parameters with the corresponding species genome as reference set, to identify overrepresented categories (Huang, et al., 2007). Gene ontologies were retrieved from Entrez and gene descriptions for yeast genes from Saccharomyces Genome Database (SGD; http://yeastgenome.org/; Cherry, et al., 2012).

4.2.8 Combining p-Values

To judge the overall significance of a series of experiments, the p-values were combined via two different approaches.

The first method is an analytic solution of the Fisher's test (Fischer, 1932). Briefly, the set point whose probability is equal to that of a set of p-values is the hyperbola:

```
(P1 · P2 · P3 · ... · Pn) = k
```

k is the product of a set of p-values k = (x1 · x2 · x3 · … · xn), provided that the events described by these p-values are independent of one another. The volume under the surface gives the probability of obtaining a set of p-values as extreme or more extreme than the given set:

```
k - (k·ln(k))
```

The alternative approach is the Z-method as described in (Whitlock, 2005), which gives similar, but somewhat more stringent results.

p-values were corrected for multiple hypothesis testing with the Benjamini-Hochberg method and the corrected values were denoted as q-values (Benjamini & HochBerg, 1995).

4.2.9 Lifespan and Vacuolar Morphology Analyses

The replicative lifespan of yeast mutants in the BY4742 (Mat α; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0) background were measured on different media as described before (Tang, et al., 2008). The *ad libitum* (AL) medium was YEPD: yeast extract (1%), peptone (2%), agar (2%), and D-glucose (2%). The dietary restricted (DR) medium was yeast extract (1%), peptone (2%), agar (2%), and D-glucose (0.5%). For bud counting, cells were grown at 30°C and dissected every 100 min. Five to six rounds of bud counting were performed each day. The ageing assay plate was saved at 4°C for overnight and the bud counting was continued the next day till all cells died, which was diagnosed by either cell lysis or no budding in 2 days. The vacuolar morphology of yeast mutants under AL and DR were measured by FM4-64 labelling and chasing (Wang, et al., 1998). Briefly, cells were inoculated into AL or DR liquid media (AL: yeast extract (1%) peptone plus (2%) D-glucose; DR: yeast extract (1%) peptone plus (0.5%) D-glucose) and incubated on a shaker (150 rpm) at 30°C overnight. An aliquot of 250 µl of cells were labelled with 6 µl of 2 mg/ml FM4-64 for 1 hour. After washing off the dye, cells were chased in the corresponding medium for 3 hours and then photographed.

Significance of mean lifespan changes were assessed with the log-rank test (a.k.a. Mantel-Cox test) between mutants and wild-type as well as between dietary regimes for each genotype.

4.2.10 RNA Isolation and Microarray Analyses

Wild-type yeast cells (BY4742) were inoculated into AL or DR liquid media and incubated overnight on a shaker. These seed cultures were then inoculated into fresh media to an initial OD600 of 0.01. Cells were grown at 30°C for 12 hours. The final OD600 of cultures were 0.85-0.99. Three OD600 units of cells were collected and lysed with zymolyase. Total RNA was isolated using the Qiagen RNeasy mini kit as described in (Gebre, et al., 2012). Total RNA obtained was between 60 to 100 µg. RNA samples were processed and hybridized with GeneChip Yeast genome 2.0 arrays (Affymetrix Inc., Santa Clara, CA) by Expression Analysis (Durham, NC). The hybridization signals were normalized and analysed with the Affymetrix statistical algorithms by Expression Analysis. By a two-fold cut-off, out of 5716 probed genes 2587 (45.26%) were differentially expressed, with 1413 genes (24.72%) upregulated and 1174 genes (20.54%) downregulated. Full results and raw microarray data are available online at GenDR (http://genomics.senescence.info/diet/Yeast array.zip), GEO (GSE38635), and ArrayExpress (E-MTAB-1165).

For additional microarray data from yeast (GSE9217), worms (GSE9682), and flies (GSE26726 and GSE16738), GSE files were retrieved from the GEO database, replicates were averaged and fold changes calculated via custom Python scripts.

For the microarray data from worms and flies, GSE files were retrieved from the GEO database, replicates were averaged and fold changes calculated via python scripts (GSE9217 (different levels of glucose for yeast), GSE9682 (intermittent fasted nematodes), GSE18563 (glucose restricted nematodes), GSE6057 (nematodes in CeMM), GSE26726 (fly transcriptional) and GSE16738 (fly translational)).

4.2.11 Transcription Factor Identification

Transcription factor-target gene interactions were retrieved from YEASTRACT (http://yeastract.com/). Transcription factors interacting with DR-essential genes were identified and ordered by their specificity or binomial p-value of the ratio of regulated genes with more than two-fold differentially expression upon DR relative to the total number of genes regulated by the same transcription factor. The whole genome (sacCer3) was retrieved from UCSC and regulatory regions (+500 bp sequences from the transcription start site) annotated. Transcription factor binding sites (TFBS), i.e. motifs, were integrated from SGD, YEASTRACT, and YFTD (http://biochemie.web.med.uni-muenchen.de/YTFD/YTF alpha 2.htm), as well as from the literature. Via regular expression it was tested (hypergeometric p-value and FDR q-values) whether a defined motif is significantly enriched in the regulatory regions of differential expressed (either up- or down-regulated) genes or set of genes.

The +500 bp sequences of yeast genes, +1500 bp to -500 bp for murine genes and +1000 bp to -500 bp for human genes, relative to the transcription start site were retrieved from Ensembl. Whole genomes (sacCer3, ce6, dm3, mm9, hg19) were retrieved from UCSC via worldbase.

4.2.12 Chromatin Mark Enrichment/Depletion

Data about chromatin modifications (histone acetylation and methylation) were obtained from (Kurdistani, et al., 2004; Pokholok, et al., 2005; Xu, et al., 2005). Differentially expressed genes (> 2-fold) were assigned to their value for each chromatin mark. A binomial test between the average upregulated and downregulated genes as well as between the average differentially expressed and non-differentially expressed genes was performed. The signal strength for all differentially expressed genes, upregulated genes, downregulated genes and all other were collected. Then the average values (actually the lists which give raise to these numbers) of them were compared with a binomial test in order to find which marks are enriched in up vs. down and delta vs. other.

4.2.13 Interaction Integration

Via a python script for each dataset, gene/protein names/identifiers, experimental system type (genetic or physical), interaction type (e.g. association, complex formation, etc.) experimental system (e.g. yeast-two hybrid), post-translational modifications events (e.g. phosphorylation), pmids, and source databases were extracted and first converted to a unified schema (unique_id_a, unique_id_b, experimental_system_type, interaction_type, experimental_system, modification, pmid, taxid, source_database), sorted according to taxonomy identification (taxid) and then subsequently fused.

4.2.14 Chance of Differentially Expression

Whether DR-essential genes are more likely to be differentially expressed than expected by change was calculated by the hypergeometric test. In mammals a signature derived from genome-wide meta-analysis of DNA microarrays (Plank, et al., 2012) was employed.

4.3 Results

4.3.1 Construction of GenDR, a Database of DR-Related Genes

A list of DR-essential genes was compiled from the literature. A DR-essential gene was defined as one which when genetically manipulated in a given organism blocks or disrupts the life-extending effect of DR. This criterion applies even if it was shown for only a single DR regimen. Over 100 genes were identified, mostly from the traditional biomedical model organisms [Table 3: Number of DR-Essential Genes and Orthologs in GenDR]. In mice, for example, one DR-essential gene found so far is the growth hormone receptor (*Ghr*) gene of which homozygous knockout mutants were long-lived and DR failed to further extend their lifespan (Bonkowski, et al., 2006). In the case of yeast, DR-essential genes were annotated separately for replicative and chronological lifespan. However, both were combined for the analyses presented below, in order to focus on mechanisms universal to DR. For each gene, its orthologs in other model organisms and in humans (if any) were retrieved from public databases (see Methods).

A publicly accessible database of DR-essential genes and their orthologs, called GenDR, was created which offers an important new tool to researchers working on the genetics of DR and of ageing. Although other studies have emphasized the importance of genes that disrupt life-extending effects of DR (Bishop & Guarente, 2007; Fontana, et al., 2010; Gems, et al., 2002), to the current knowledge this is the first compilation of a list of such genes. The GenDR database employs the same system and interface as the widely used GenAge database of ageing-related genes (de Magalhaes, et al., 2009b). My inclusive selection criteria allow GenDR to incorporate a broad range of genes. Because information on each gene and reason(s) for its selection are included, however, researchers are able to focus on subsets of the database that are most relevant to their work. GenDR will be useful both as an informational website and as a research tool, and it is freely available online for the research community to use (http://genomics.senescence.info/diet/).

Table 3: Number of DR-Essential Genes and Orthologs in GenDR. Number of genes reported in different model organisms to be essential for DR and their orthologs identified by the use of Ensembl, HomoloGene, InParanoid and OrthoMCL.

Species	Genes	Genes+Orthologs
Fission yeast	6	54
Budding yeast	70	170
Nematode	42	137
Fruit fly	19	187
House mouse	1	218
Norway rat	0	217
Rhesus monkey	0	200
Human	0	226

A DR-essential gene can be described by:

1. Mutation interferes with effect of DR to extend lifespan (obligatory)
2. Mutant is not sick and capable of lifespan extension (obligatory)
3. Evolutionary conserved / under evolutionary positive selection (optional)
4. Change in activity under DR (optional)
5. Interact on physical or genetic level with other DR-essential genes (optional)

Whereas only criteria number one and two are obligatory, the others are optional and are very useful to infer/identify further DR-essential genes. Gene expression profiles, interaction networks, molecular evolution data will be used to further prioritize potential DR-essential genes and identify crucial regulators. The second criteria is used to discriminate from false-positives. Mutants should still be capable exhibiting lifespan extension via other interventions which are assumed not to work via DR signalling. Thus, proving that the mutation does not just renders the animal in general sick and unable to life longer. RNAi against genes such as *pat-4* and *pat-6* abrogated DR-induced lifespan, but as the animals appeared to be very unhealthy they were not classified as DR-essential (Hansen, et al., 2005).

4.3.2 GenDR Is Enriched in Conserved Longevity Genes and Pathways

Given that DR works across diverse model organisms, it is expected that GenDR can guide the search for evolutionarily conserved longevity mechanisms. Therefore, GenDR is analysed at three levels: the sequences of genes/proteins, the interactome, and enriched pathways.

To test whether GenDR reflects the conserved effects of DR on longevity, the molecular evolution of genes essential for DR was investigated. In agreement with the hypothesis, the percentage of orthologs of DR-essential genes across all tested species is higher than expected by chance [Figure 22 DR Genes Are Molecular Conserved and Form a Tight Network A]. Likewise, the average dN/dS ratio, a measure of molecular evolution rate, as well as dN and dS rates, for DR-essential gene orthologs in mammalian species pairs were lower than expected by chance [Figure 23 DR-Essential Genes Have Low dN/dS Ratios]. As such, it appears that indeed DR is evolutionarily conserved at the genetic level.

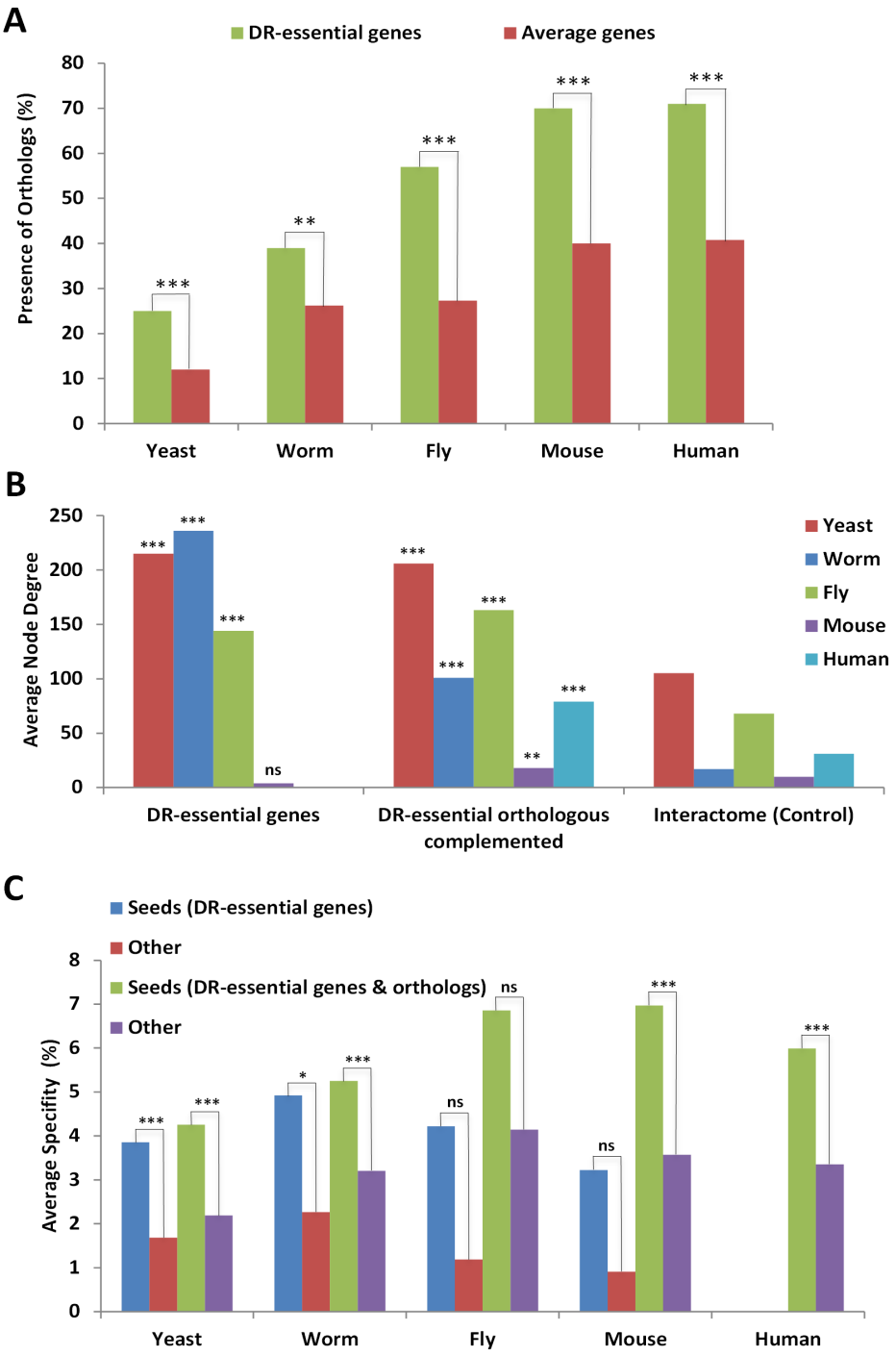

Figure 22: DR Genes Are Molecular Conserved and Form a Tight Network. *A, The proportion of genes (shown as percentages) with orthologs from multiple species in the specified organisms is in all cases higher (by 10-30%) for DR-essential genes than expected by chance. B, The average degree (number of interactions) for DR-essential genes and DR-essential gene orthologs (the ortholog-complemented set) is higher than the interactome-wide average which serves as control. C, The average percentage of specificity in interconnectivity (100× specific interactions with seed genes/all interactions) of DR-essential genes to each other is higher than for other genes in interactomes, and complementation with orthologs of DR-essential genes from other species further increases the specificity of interconnectivity. ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.*

–

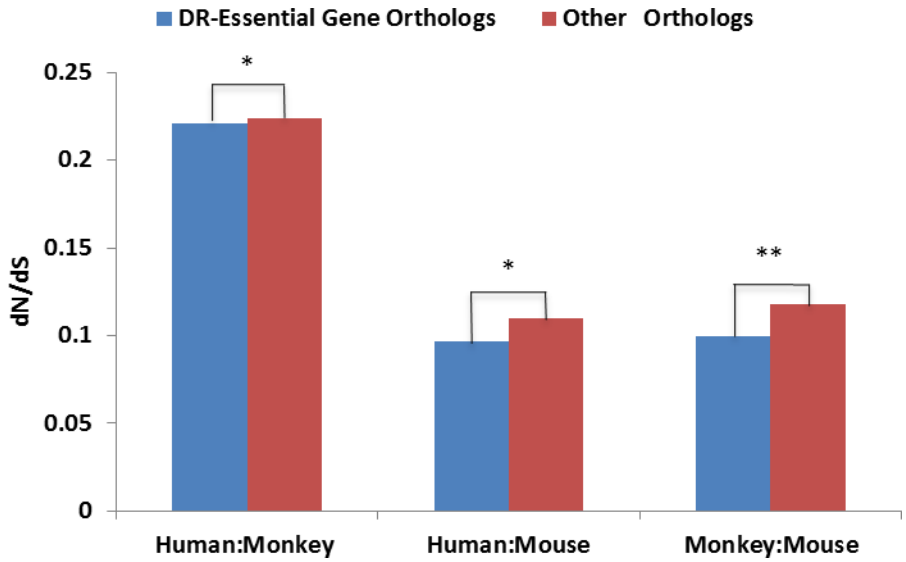

***Figure 23: DR-Essential Genes Have Low dN/dS Ratios**. Mammalian DR-essential orthologs have lower dN/dS ratio than expected by chance.*

Homology has been calculated based on sequence via the Smith and Waterman method (Smith & Waterman, 1981).

–

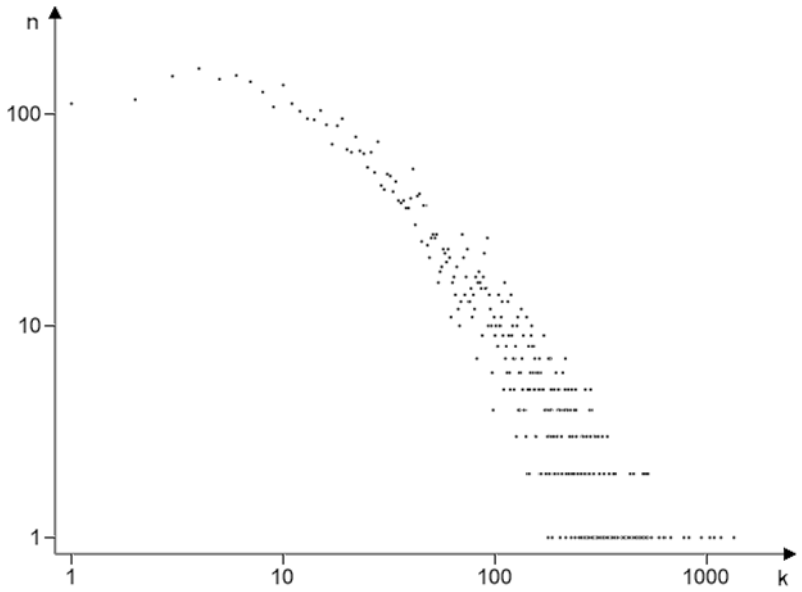

***Figure 24: DR-Essential Gene Orthologs in Humans Form a Scale-Free Network**. Degree distribution of human ortholog-complemented DR-essential gene network, as a log-log plot: i.e. log[degree (k)] is plotted against the log of the number of nodes with degree k (n).*

Interaction networks of DR-essential genes plus their orthologs were created by integrating molecular interaction information from various sources: physical, genetic and other interactions such as regulatory relationships were retrieved from public databases (see Methods). The human orthologs of DR-essential genes form an interaction network in which the total interactions appear to follow approximately a power law distribution [Figure 24 DR-Essential Gene Orthologs in Humans Form a Scale-Free Network]. This is consistent with a scale-free network in which only a few nodes (often called “hubs”) are highly connected.

In other words, a few hubs contribute highly to the DR network connectivity, as has been observed in other biological networks (Budovsky, et al., 2007; Wang, et al., 2009).

–

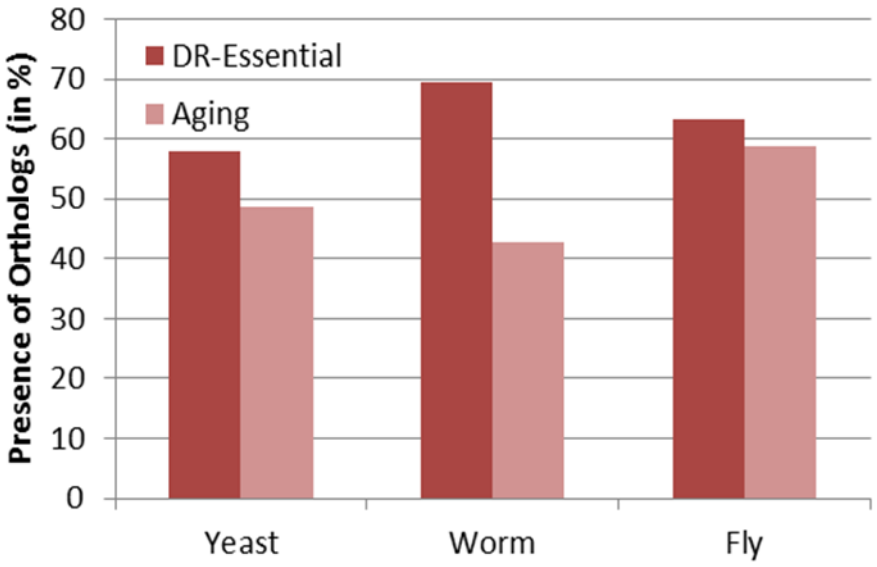

Figure 25: DR-Essential Genes Are More Conserved Than Ageing Genes*. DR-essential genes have a higher abundance of orthologs than ageing genes.*

DR-essential genes as well as their orthologs have a higher node degree than expected by chance when compared to the whole interactome [Figure 22 DR Genes Are Molecular Conserved and Form a Tight Network B]. Thus, DR-essential genes tend to be located in the centre of the interactome rather than in the periphery. As anticipated, DR-essential genes interact with each other more than expected by chance [Figure 22 DR Genes Are Molecular Conserved and Form a Tight Network C]. Adding orthologs to the DR-essential gene seed lists (orthology complementation) increased the specificity (interaction with seed genes/total interactions) of the connections between seed genes and enabled the generation of interaction networks for species with few or no known DR-essential genes, such as mammals. DR-essential as well as ageing genes are significantly enriched for signalling genes. Nonetheless, DR-essential genes are even more evolutionarily conserved than ageing genes [Figure 25 DR-Essential Genes Are More Conserved Than Ageing Genes] and have a higher average degree than signalling genes [Figure 26 DR Genes Have a Higher Node Degree Than Ageing & Signalling Genes], indicating that these properties (evolutionary conservation and high degree) are not secondary effects expected for subsets of ageing genes and signalling genes, respectively.

One issue, however, is that the finding of higher conservation and interconnectivity for DR-essential genes compared to other genes might reflect a selection bias of researchers, who tend to study genes that have orthologs in multiple species and/or those in pathways associated with DR. Because node degree, presence of orthologs and sequence conservation are thought to be related, the correlations among these variables for human genes were investigated. It was found that log(degree) and number of species with orthologs are positively correlated (Pearson correlation coefficient, $r = 0.36$; $p<1e-200$), which implies that the higher the number of connections (i.e. interactions) of a human gene the higher the number of species which have orthologs of that gene. Similarly, log(degree) was weakly negatively correlated with log(dN/dS) ($r = -0.08$; $p<1e-11$), indicating that genes with more connections have slightly lower dN/dS ratios and hence more conserved sequences. Finally, the log(ortholog number) was also found to be negatively correlated with log(dN/dS) ($r = -0.24$; $p<1e-100$), suggesting that genes which have orthologs across many species also tend to have lower dN/dS ratios, as expected. An interpretation of these results is that genes with high degree (more connectivity) tend to also have high numbers of orthologs and low dN/dS ratios. This makes sense, since important hubs in the network have more constraints limiting their evolutionary divergence. Next, multiple linear regression analysis (with log-transformed data) was performed to derive equations relating each of these three variables as a function of the other two. The equations was used to

determine whether DR-essential gene orthologs have higher or lower values than predicted from the other two variables. The results reveal that DR-essential gene orthologs have an average degree much higher than expected (73 vs. 12), a slightly higher number of species with orthologs (10 vs. 9) and dN/dS ratios higher than the expected value (0.08 vs. 0.04). Thus, a high node degree appears to be the most critical feature of DR-essential gene orthologs.

–

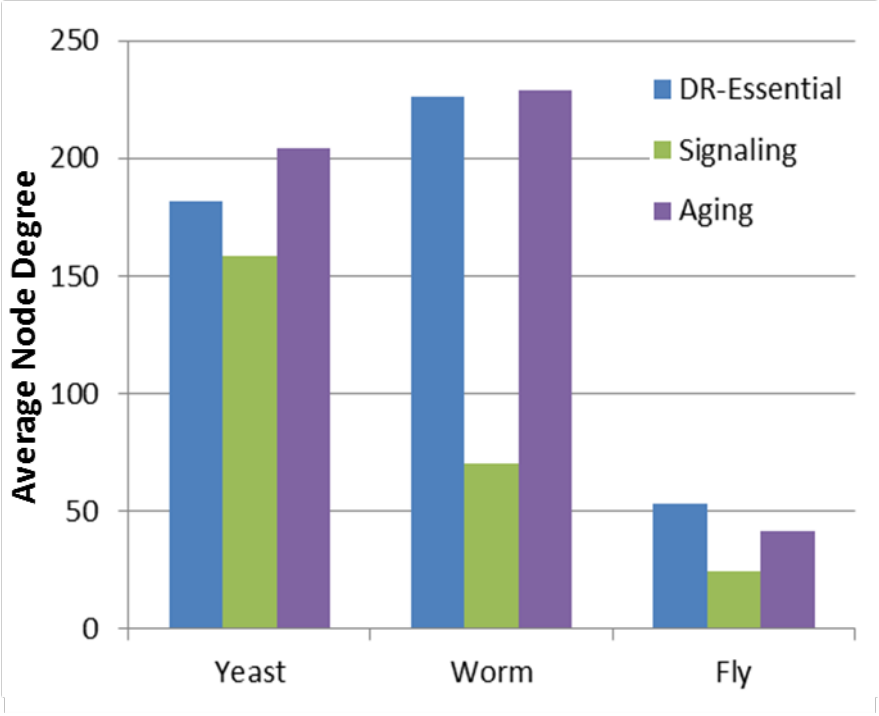

Figure 26: DR Genes Have a Higher Node Degree Than Ageing & Signalling Genes*. DR-essential genes exhibit a high average node degree relative to ageing genes or signalling genes.*

To ascertain which processes and functions are common to the DR gene network, DR-essential genes plus their significant interactors ($p<0.05$ according to specificity of interaction with DR-essential genes, as described in the Materials and Methods) for each species were subjected to functional enrichment analysis using DAVID (Database for Annotation, Visualization and Integrated Discovery). Common to all DR-essential gene interaction networks in yeast, worm and fly were the terms ageing ($p<1e-27$) and mitochondrion ($p<1e-10$). Terms common to ortholog-complemented networks in yeast, worms, flies, mice and humans were categories related primarily to phosphorylation signalling, among others. Kinase signalling and protein phosphorylation are also dramatically suppressed in very long-lived PI3K-null mutant worms (Shmookler Reis, et al., 2012; Tazearslan, et al., 2009). It was then asked which genes among the significant interaction partners were common to multiple organisms. Although no homologous group was significant at False Discovery Rate (FDR) < 0.05 (which appears to be too strict for comparisons across multiple species), by the more relaxed criterion of having a binomial p-value < 0.05, four homologous groups were nominally significant: *CAB39*, the genes encoding 14-3-3 proteins, sirtuins, and AHCY encoding S-adenosylhomocysteinase.

4.3.3 Guilt-by-Association Discovers Novel Vacuolar DR-Essential Gene Functions

To predict novel DR-essential candidate genes a guilt-by-association strategy was used (de Magalhaes & Toussaint, 2004). Essentially, the logic behind this approach is that a gene with more interactions than expected by chance with genes associated with a given process (i.e. DR-mediated lifespan extension) is likely to also play a role in that process (see Methods). The candidate genes with highest significance in their connectivity to DR-essential genes were *YGR272C* (*EFG1*; Exit From G1) in *Saccharomyces cerevisiae*, *B0280.10* (*pot-1*; Protection of Telomeres 1 (Pot1) homolog) in *Caenorhabditis elegans*, and *Akt1* (serine-threonine kinase involved in insulin-like signalling) in *Drosophila melanogaster*. After orthologous complementation, among the highly significant candidate genes were, for example, *PAI3* (essential Proteinase A inhibitor) in *S. cerevisiae*, *hcf-1* (human host cell factor (HCF-1) homolog) in *C. elegans*, *CG7333* (an organic cation transmembrane transporter) in *D. melanogaster*, *Raf1* (MAP kinase kinase kinase) in *Mus musculus*, and ACD (involved in telomere maintenance and meiosis) in humans. In yeast, worm, and fruit fly, respectively, 372, 1317, and 202 genes were significant at a p-value < 0.05. After orthologous complementation there were 264 genes significant in yeast, 228 in worm, 743 in fruit fly, 2576 in mouse, and 996 in humans.

Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes. Vac = Vacuole; r = round; f = fragmented. Ratio = Ratio of mRNA levels (DR/AL) in WT.

Mutant	GbA p-value	Mean / max. AL lifespan, (N)	Mean / max. DR lifespan, (N)	AL, mutant vs. WT, log-rank p-value	DR vs. AL, log-rank p-value	Vac AL	Vac DR	Vac Δ	Ratio
wild-type	NA	26.4/47 (51)	31/54 (50)	NA	0.002	r	r	NA	NA
vps20Δ	0.02	22.07/38 (30)	18.27/34 (30)	0.09	0.052	NA	NA	NA	1.56
fre6Δ	0.022	30.07/46 (30)	28/42 (30)	0.043	0.173	r	f	29 -> 14	0.85
rcr2Δ	0.019	31.27/45 (30)	24.96/45 (25)	0.008	0.048	r	f/r	52 -> 18	3.22*
ydl180wΔ	0.043	27.67/44 (30)	28.2/46 (25)	0.262	0.596	f	f/r	NA	2.25*
opt2Δ	0.048	32.6/51 (15)	25.43/39 (30)	0.007	0.015	f	f	NA	0.05*
gtr1Δ	0.037	16.93/23 (15)	18.73/29 (15)	4.50e-9	0.208	r	f	NA	2.77*
dap2Δ	0.013	21.27/30 (15)	19.93/32 (15)	0.001	0.969	r	f	60 -> 1	1.22
slm4Δ	0.049	23.87/48 (15)	23.4/36 (15)	0.793	0.597	r	f	44 -> 16	3.28*
yol092wΔ	0.043	20.8/34 (15)	27.4/42 (15)	0.087	0.062	f	f	NA	0.33*

–

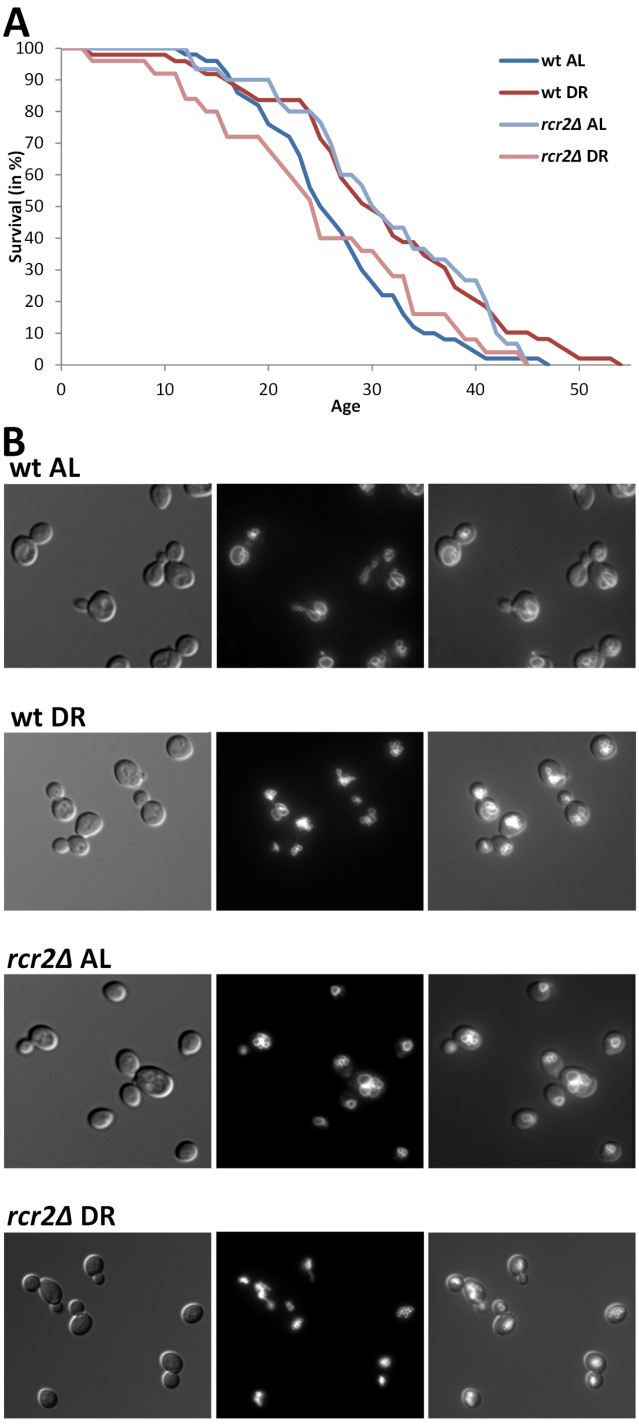

Figure 27: DR Vacuole-Associated Genes Are Required for DR Lifespan Extension and Normal Vacuolar Morphology*. A, Survival curves of wild-type and rcr2Δ mutant strains measured on AL/YEPD and DR media. The units of age (X axis) are generations. Mean and maximum lifespans as well as sample sizes are listed in [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes. B, Vacuolar morphology on AL and DR media of wild-type and rcr2Δ. Left panel: DIC images showing the cells; middle panel: TRITC-fluorescent images showing the vacuoles; right panel: overlay of cell and vacuole images.*

In order to assess the sensitivity and specificity of this guilt-by-association concept, a leave-one-out test was applied (Owen, et al., 2003). By omitting one of the DR-essential genes from the seed list and checking whether the removed gene was among the significant candidate genes (i.e. predicted correctly), a measure was obtained for sensitivity of the guilt-by-association method in the form of the percentage of genes which were recovered (i.e. rediscovered). This method achieved a recovery rate of 40% in yeast, 12% in worm and 50% in fruit fly. The lower recovery rate for worms compared to the other species (and the higher p-values) is possibly because *C. elegans* had the lowest number of known interactions at the time of analysis.

To test the new candidate genes, and thus to validate the usefulness of GenDR and DR network analyses as tools for ageing research, the role in DR of some of the yeast candidates were experimentally assessed. Vacuole-related terms were among the most significant discrete clusters of terms in the DR-essential network of yeast (Enrichment Score: 5.2; q-value < 2×1e-5). The guilt-by-association method predicted nine yeast genes whose protein products are on the vacuolar membrane and are non-essential for viability. Therefore, it was decided to focus on the mutants of these genes, and let the replicative lifespan and vacuolar morphology of these nine mutants on AL and DR be measured [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes; Figure 27 DR Vacuole-Associated Genes Are Required for DR Lifespan Extension and Normal Vacuolar Morphology]. Eight of the nine mutants that were analysed exhibited an impaired lifespan extension by DR (i.e. are DR-essential genes). Five of the nine mutations altered the extent of DR-induced vacuole fragmentation. While the vacuolar morphology of wild-type and rcr2Δ are indistinguishable in AL, their morphologies in DR media are different; wild-type had some cells with a few vacuolar vesicles (equal or less than 5/cell) but rcr2Δ cells had 6 or more vacuolar vesicles/cell [Figure 27 DR Vacuole-Associated Genes Are Required for DR Lifespan Extension and Normal Vacuolar Morphology]. Similar to mutants (erg6Δ, nyv1Δ, etc.) with highly fragmented vacuoles under DR (Tang, et al., 2008), rcr2Δ had a shortened lifespan on DR [Figure 27 DR Vacuole-Associated Genes Are Required for DR Lifespan Extension and Normal Vacuolar Morphology]. Deletion of *OPT2* or *FRE6* showed results similar to rcr2Δ and all had an extended lifespan on AL [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes].

Further DR-essential genes in mouse [Figure 28 M. musculus DR-Essential Gene Network], fly [Figure 29 D. melanogaster DR-Essential Gene Network], nematode [Figure 30 C. elegans DR-Essential Gene Network], and yeast [Figure 31 S. cerevisiae DR-Essential Gene Network] are predicted as well as significantly associated processes, functions, locations, and pathways.

Guilt-by-association was used to generate the networks with q-value threshold of 0.05 for the respective species. The shortest path algorithm was used to connect unconnected nodes. Processes are coloured green, functions are orange and locations are yellow. This results in networks of physical and genetic interaction as well as functional edges of DR-essential genes and their significant associations (q-value < 0.05). Nodes representing DR-essential genes are golden. Processes are light green, functions are orange, locations are yellow, and pathways are white. Physical interactions are pink, genetic interactions are green and other edges like, *is involved in*, *participates in*, *exhibits* and *is located in* are grey. Node size is proportional to the 10+log10 of the q-value of being associated with DR-essential genes.

–

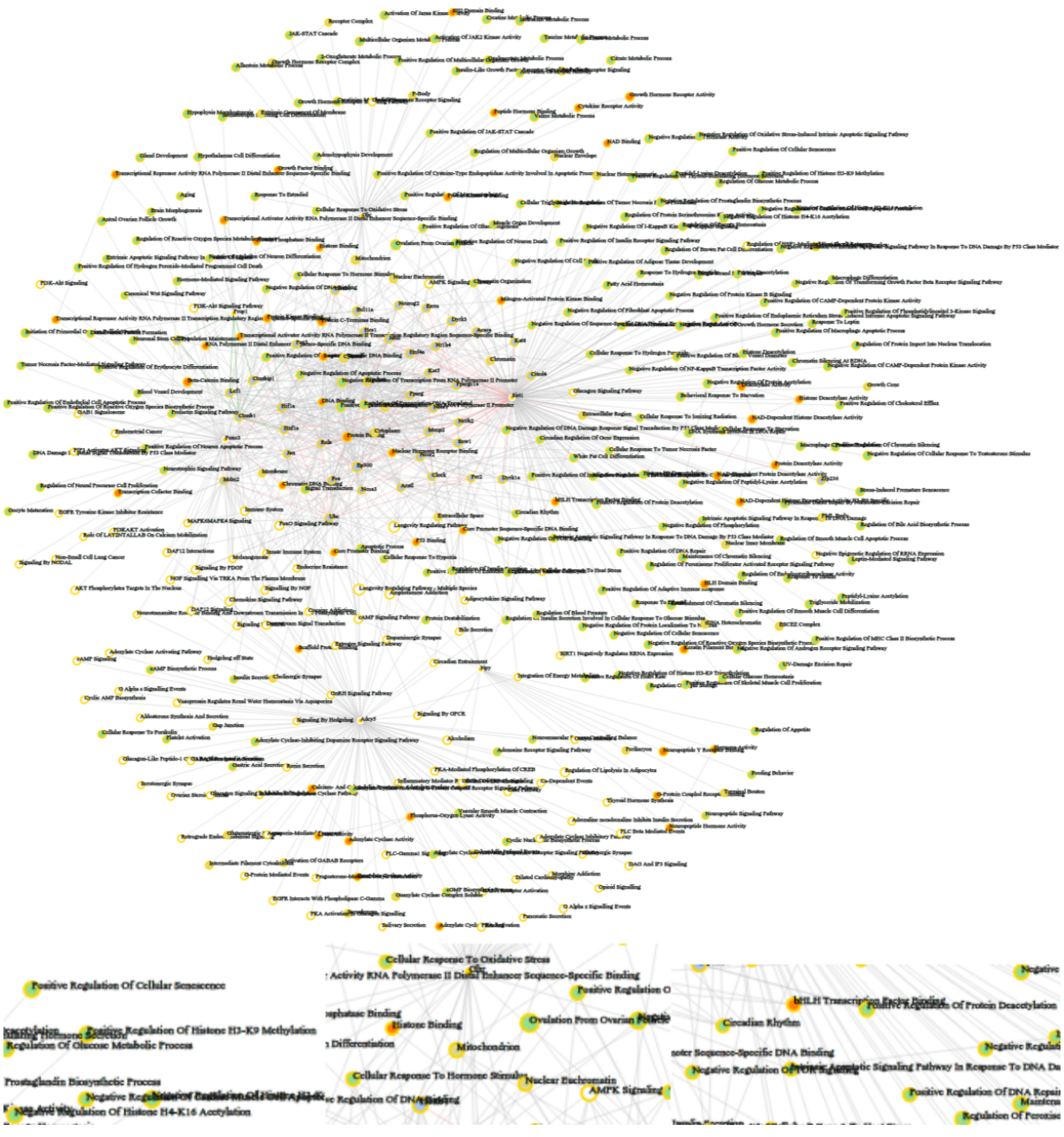

Figure 28: M. musculus DR-Essential Gene Network.

Mouse DR-essential genes are significantly associated with physical interaction with Ep300, genetic interaction with Cby1, involved in epigenetics, exhibit beta-catenin binding, and located in growth hormone receptor complex.

–

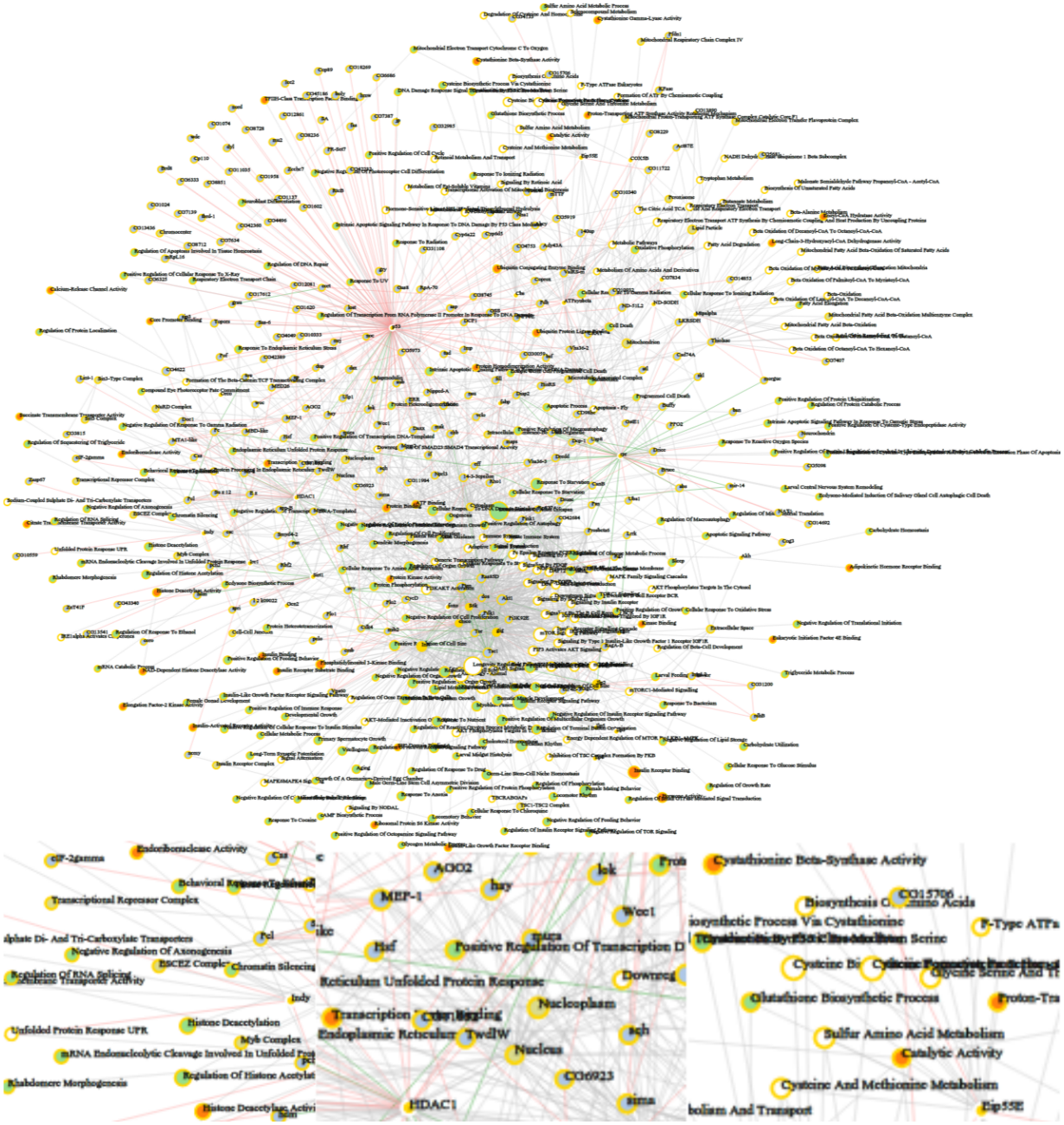

Figure 29: D. melanogaster DR-Essential Gene Network.

Fly DR-essential genes physically interact with InR, genetically interact with Akt1, involved in lifespan control, participate in longevity, exhibit deacetylase activities, and being located in TSC1-TSC2 complex.

–

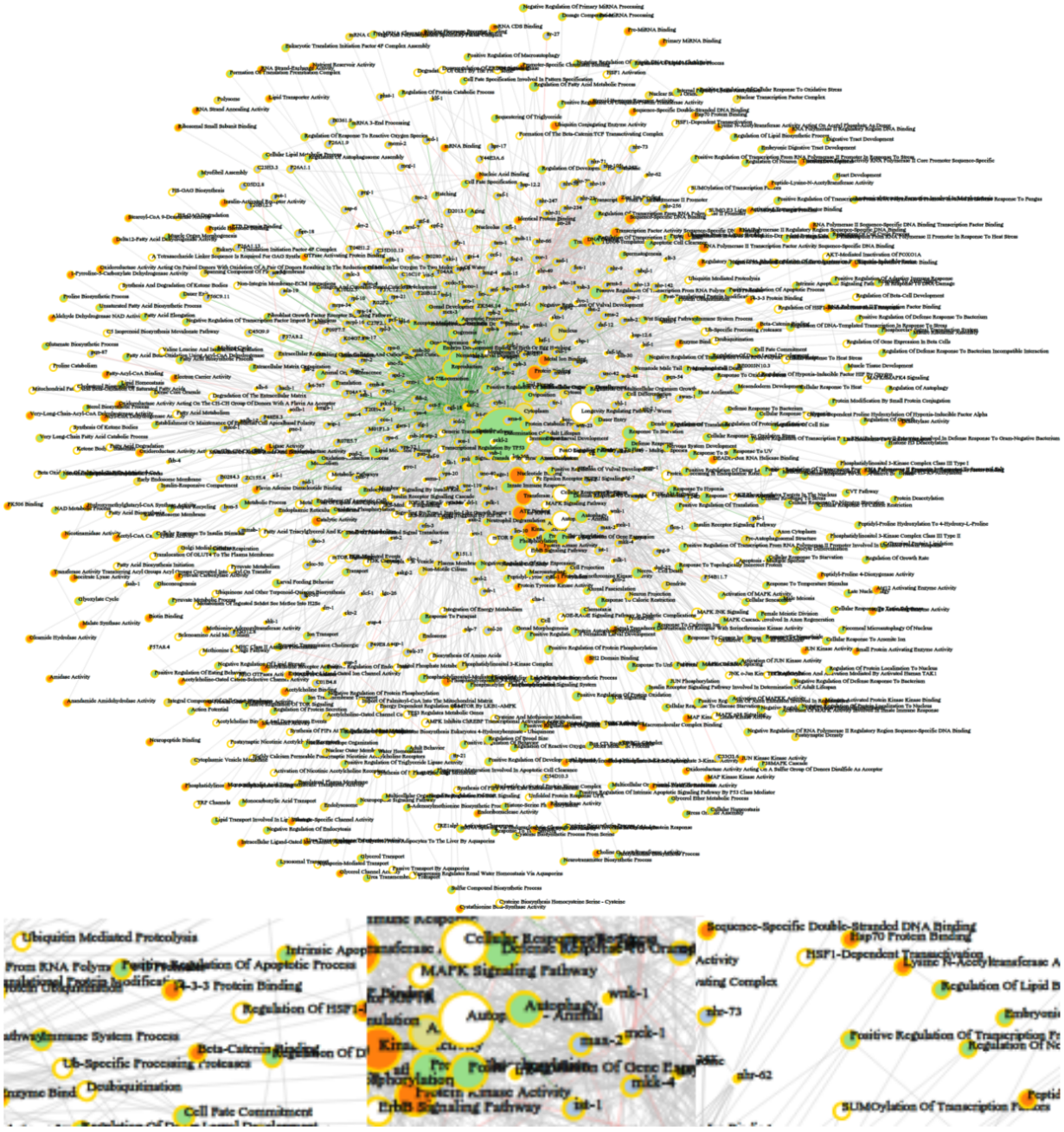

Figure 30: C. elegans DR-Essential Gene Network.

Worm DR-essential genes are significantly associated with physical interaction with col-20, genetic interaction with bar-1, involved in ageing/lifespan/senescence, exhibiting MAPK-related activities, and being located in insulin-responsive compartment.

–

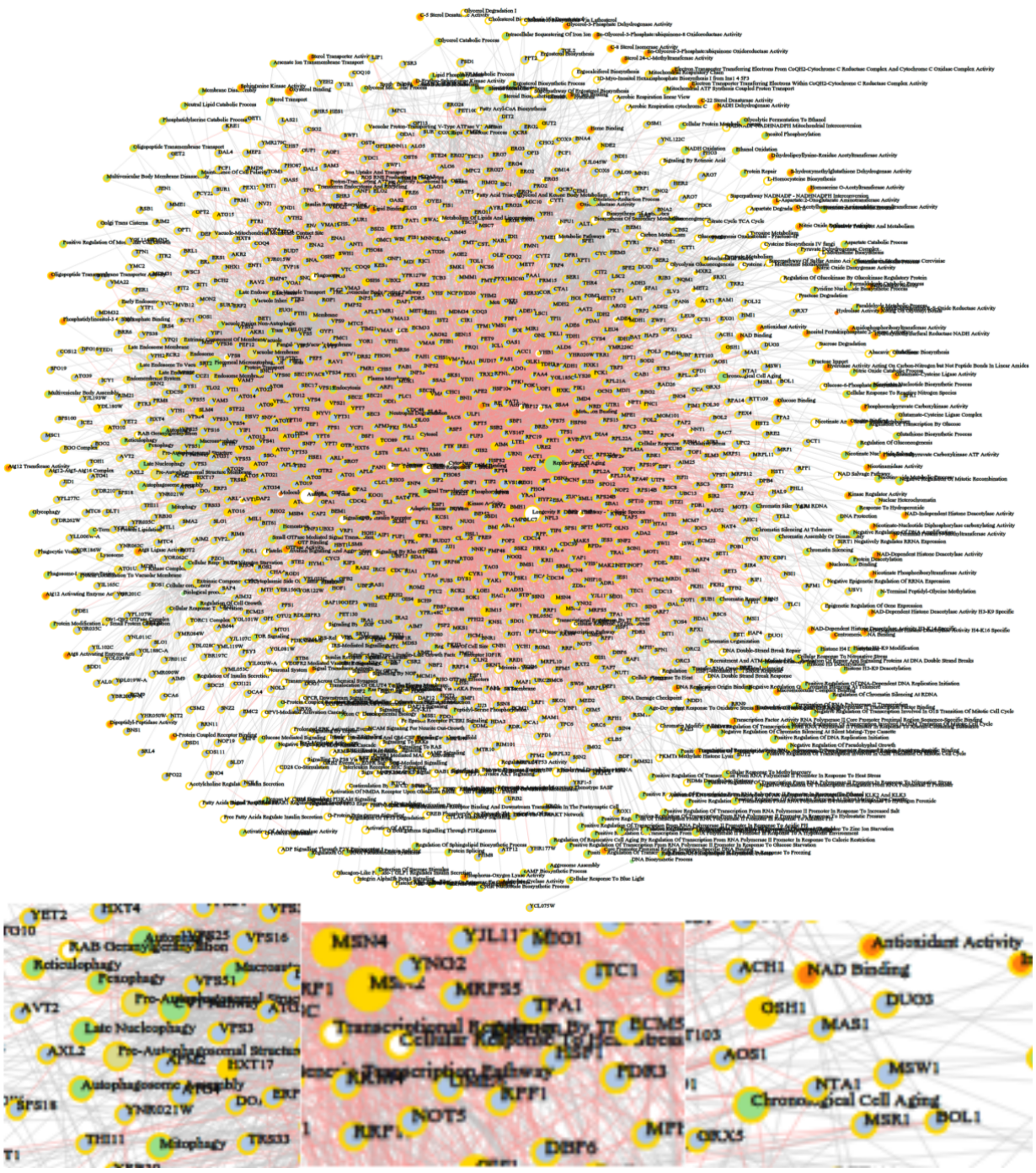

Figure 31: S. cerevisiae DR-Essential Gene Network.

Yeast DR-essential genes physically interact with VPS21, genetically interact with SCH9, participate in longevity regulating pathway - multiple species, exhibit NAD-dependent histone deacetylase activity, and are located in fungal-type vacuole membrane.

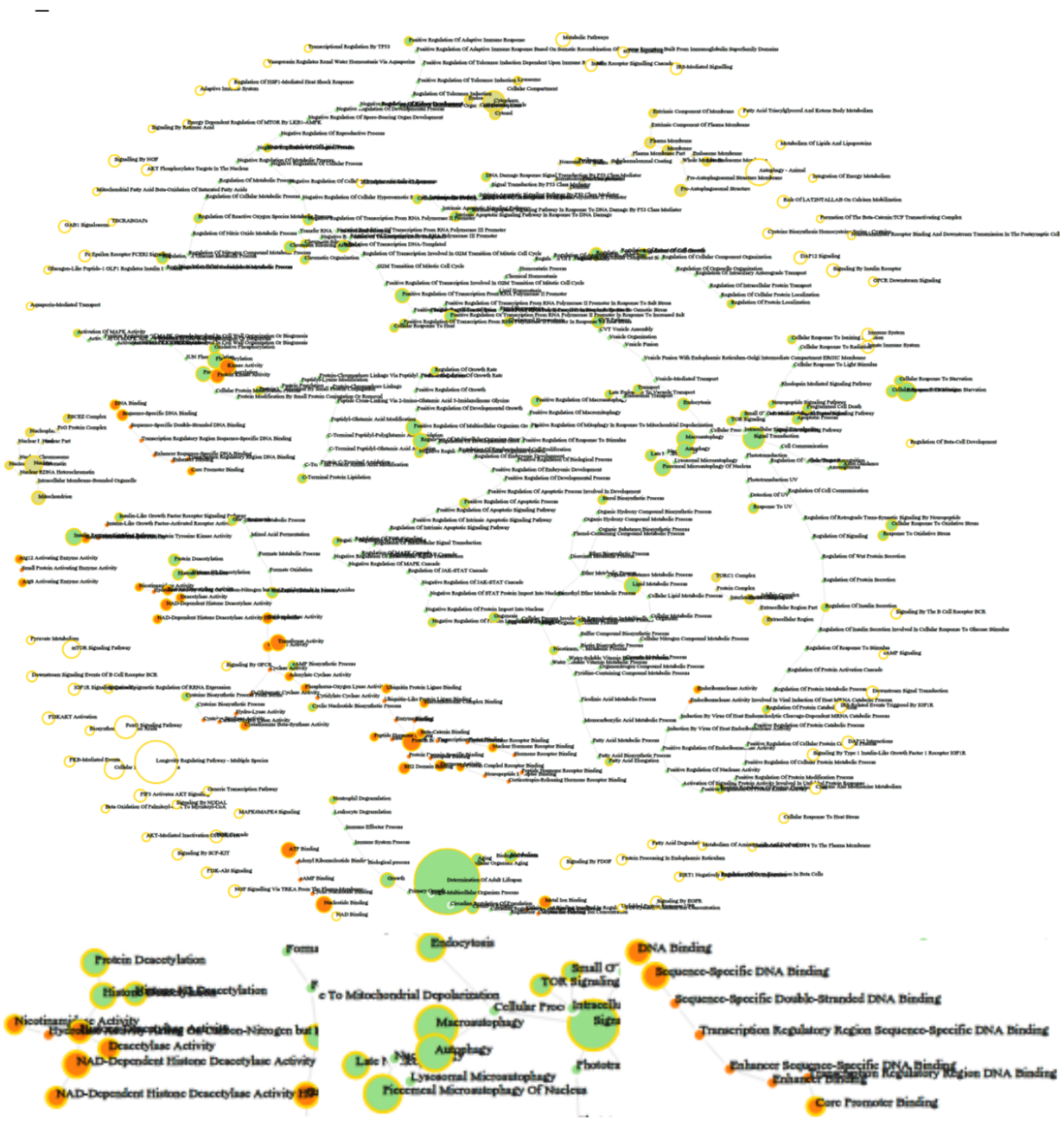

Figure 32: Network of Associations Common to DR-Essential Gene Across Species.

Next nodes that are commonly significant in multiple (two or more) species by a common relaxed threshold of 0.05 were identified and graphed [Figure 32 Network of Associations Common to DR-Essential Gene Across Species].

DR-essential genes are commonly significantly associated with ageing/lifespan/longevity, IIS, TOR, MAPK, and Wnt.

4.3.4 Differential Expression of DR-Essential Genes upon DR

For the purpose of gaining insights into which processes on the global scale are affected by DR, microarray data was generated in yeast under DR (see Methods) and those genes more than two-fold differentially expressed were examined using DAVID by retrieving terms and clusters with FDR < 5%. DR up-regulated genes were associated with heat response, mitochondria, peroxisome, transcription, mRNA processing, zinc binding, carbohydrate metabolism, sporulation, vacuole, and mitochondrial ribosomes, while DR down-regulated genes were related to ribosome/translation, nitrogen, sterol and one-carbon metabolism as well as DNA replication.

This comparison of mRNA levels between yeast cells cultured in AL and DR media, as ascertained on microarrays, also furnished further support for the involvement of the predicted vacuolar DR-essential genes. Of the 9 predicted vacuolar DR-essential genes, 6 were differentially regulated by more than two-fold either up or down [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes. Strikingly, *OPT2* which had the strongest effect on lifespan when deleted (around 30% mean and maximum lifespan extension), was also among the genes most strongly (20-fold) down-regulated by DR [(Lin, et al., 2002) and DNA microarray data generate in this study here; see Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes]. OPT2 is differential expressed in numerous intervention which impact on lifespan (Aghajan, et al., 2010; Chattopadhyay, et al., 2009; Lin, et al., 2002; Reinke, et al., 2006).

As changes in expression of DR-essential genes via genetic manipulations mimic and/or abolish the lifespan extension conferred by DR, one interpretation is that DR-essential genes normally mediate DR effects by changing their expression or activity level in response to DR. As an approximation to investigate this, large-scale microarray expression profiles of yeast, worm (Honjoh, et al., 2009) and fly (Bauer, et al., 2010; Zid, et al., 2009) was employed. Numerous DR-essential genes change in their activity either at the transcriptional or translational level in response to DR. Of note among genes differentially expressed during DR, only two DR-essential homologous groups are shared in common by yeast, worm and fly: homologs of a fatty acid elongase, and S-adenosylmethionine synthetase activity. Experimental data support the importance of these two categories for which GenDR and DR-induced differential expression agree: (1.) worms with RNAi-suppressed fatty acid elongase activity have increased lifespan, and long-lived *C. elegans* mutants tend to have shorter fatty acid chains in proportion to their longevities (Shmookler Reis, et al., 2011); and (2.) knockdown of the nematode SAM synthetase gene, *sams-1*, extends adult lifespan (Hansen, et al., 2005; Steinkraus, et al., 2008b), and also in yeast *SAM1* deletion extends lifespan (Smith, et al., 2008). However, DR-essential genes in yeast are no more likely to be differentially expressed than expected by chance (30 of 70 DR-essential genes among the DR-differentially expressed genes; hypergeometric p-value = 0.6) at a threshold of two-fold change.

4.3.5 Comparing DR Interactomes

The validation of vacuole mutants and the responses of DR-essential genes to longevity manipulations support the biological relevance of predictions based on the DR interactome. To further study conserved mechanisms of DR-induced life-extension, next interactome and transcriptome data were integrated and analysed [Figure 33 Comparative Interactomics of DR]. The interactomes of yeast, worm and fly were integrated with their respective DR triggered gene expression changes and condensed interaction networks were generated via the ExprEssence algorithm (Warsow, et al., 2010). These networks were further condensed until the representative graph contained approximately 1,000 genes. Two graphs for each species were created, one containing only startups (induced interactions), while the other contained only shutdowns (suppressed interactions), thus partitioning the interactions of up- and down-regulated genes, respectively.

Next, DAVID was used to retrieve the functionally enriched terms for each graph, retaining only the terms common to either all startups or all shutdown networks which surpass a q-value < 0.05. Subsequently, p-values were combined across all three species (Table 5: Changed Interactions by DR Common to Invertebrates; see Methods). Suppressed interactions were enriched for terms associated with translation such as ribonucleoprotein, structural molecule activity and ribosome. This is consistent with previous observations that downregulation of translation or ribosomes extends lifespan in diverse organisms from yeast to mammals (Steffen, et al., 2008; Hansen, et al., 2007; Kapahi, et al., 2004; Selman, et al., 2009).

–

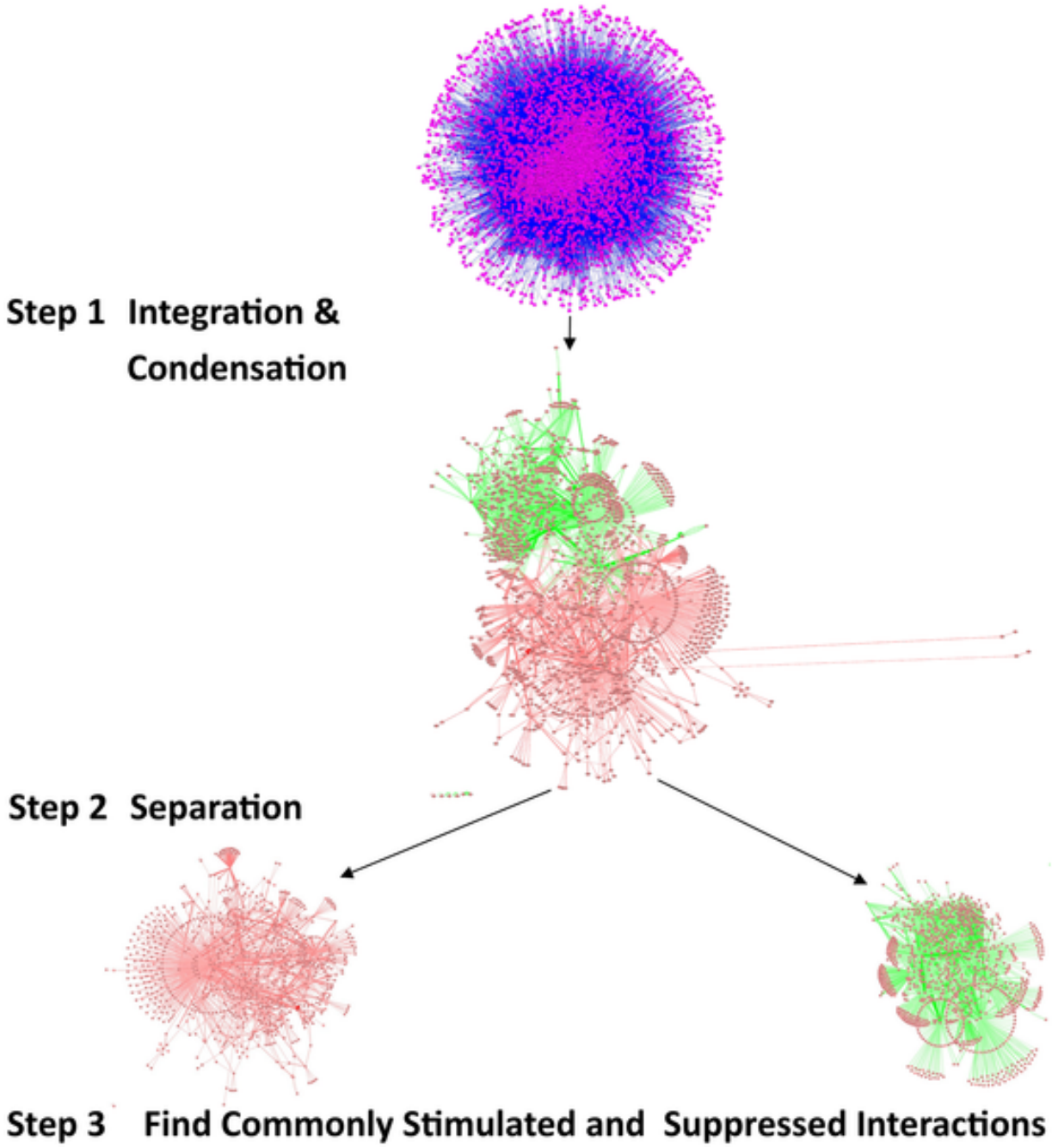

***Figure 33: Comparative Interactomics of DR**. Comparing the influence of gene expression changes upon DR, in multiple interactomes. The interactomes of yeast, worm, and fly were integrated with gene expression information upon DR and condensed interaction networks generated via the ExprEssence algorithm (Step 1). Interaction networks restricted to either suppressed or stimulated interactions for each species were created (Step 2). The suppressed and stimulated networks were then separately compared for common significant functional enrichments (Step 3). Functional terms which were common to suppressed and induced interactions upon DR in yeast, worm, and fly are listed with their respective p-values [Table: 5 Changed Interactions by DR Common to Invertebrates; Table: 6 Changed Interactions by DR Common to Invertebrates and Mammals].*

Stimulated interactions mostly occurred between genes involved in modifying chromatin structure such as chromatin regulator, chromatin modification, and chromosome organization, genes associated with

reproductive developmental/cellular processes as well as cell cycle and stress response. Strikingly, the majority of these genes associated with cell cycle are involved in meiosis with p-values of <1e-19, <1e-46, and <1e-5 for yeast, worm, and fly, respectively. In accordance with the functional enrichment analysis of DR-essential networks, the comparative interactomics changes suggest that DR both stimulates and suppresses interactions associated with phosphoproteins and nucleus [Table 6: Changed Interactions by DR Common to Invertebrates]. To unravel the relationship between these two broad terms all kinases and phosphoproteins in yeast known to be localized in the nucleus were identified (using Gene Ontology annotation), that are either up- or down-regulated upon DR. The induced and suppressed nuclear kinases are significantly enriched for meiosis (each q-value < 1e-4). While most of the induced nuclear kinases are positive regulators, the suppressed nuclear kinases are mainly negative regulators of meiosis, indicating that DR in yeast promotes the initiation of meiosis. Interestingly, yeast genes associated to meiosis, according to Gene Ontology (161 genes), had an overall average gene expression increase of 2.7 upon DR (higher than expected by chance; Mann Whitney U test p-value = 0.002) and together with all their interaction partners were on average two-fold upregulated (Mann Whitney U test p = 0.003). Induced nuclear phosphoproteins (i.e. substrates of kinases) are enriched for protein phosphatases (q-value < 1e-23), ER-nuclear signalling (q-value < 1e-4), MAPK signalling, Mn (q-value < 1e-4), sexual reproduction/reproductive cellular process (q-value < 6e-3/0.03), while suppressed nuclear phosphoproteins were only enriched for protein phosphatases (q-value < 1e-10).

Table 5: Changed Interactions by DR Common to Invertebrates. Induced and suppressed interactions common to yeast, worm and fly. The functional enriched terms for either up- or downregulated interactions associated to the DR-induced gene expression changes of multiple species (budding yeast, nematode, fruit fly) are listed.

Terms associated to stimulated interactions	p-value	Terms associated to suppressed interactions	p-value
phosphoprotein	1.55e-51	phosphoprotein	5.26e-97
nucleus	1.72e-46	ribonucleoprotein	5.15e-87
nucleotide-binding	5.61e-42	ribonucleoprotein complex	1.55e-86
ATP-binding	1.48e-39	cytoplasm	7.21e-74
cell cycle	5.82e-36	nucleus	1.54e-45
kinase	5.5e-35	nucleotide-binding	2.46e-42
serine/threonine-protein kinase	9.92e-33	ribosome	3.68e-37
cell cycle process	6.53e-30	RNA-binding	7.55e-32
reproductive developmental process	1.65e-22	structural molecule activity	3.92e-28
chromatin regulator	5.85e-14	ATP-binding	6.94e-27
chromatin modification	1.35e-13	ATP	9.92e-17
chromosome organization	3.07e-13	nucleotide binding	4.7e-09
ATP	1.92e-11	ubl conjugation	9.5e-09
reproductive cellular process	4.51e-11		
repressor	1.33e-08		
coiled coil	2.82e-08		
cellular response to stress	3.88e-08		
cellular protein catabolic process	6.82e-08		
negative regulation of gene expression	4.17e-07		

Table 6: Changed Interactions by DR Common to Invertebrates and Mammals. Induced and suppressed interactions common to yeast, worm, fly and mammals. The functional enriched terms for either up- or downregulated interactions common to the DR-induced gene expression changes in budding yeast, nematode, fruit fly and mammals (meta-analytic signature).

Common terms of induced interactions	p-value	Common terms of suppressed interactions	p-value
phosphoprotein	7.30e-64	phosphoprotein	2.32e-116
nucleotide-binding	3.51e-50	non-membrane-bounded organelle	2.49e-89
ATP-binding	1.31e-45	intracellular non-membrane-bounded organelle	2.49e-89
active site:Proton acceptor	4.27e-32	cytoplasm	4.85e-84
reproductive developmental process	8.89e-30	nucleotide-binding	1.14e-54
transcription regulation	1.88e-24	nucleus	6.20e-53
transferase	6.69e-23	regulation of cellular protein metabolic process	4.92e-46
phosphotransferase	7.58e-17	RNA-binding	2.00e-37
negative regulation of macromolecule metabolic process	2.40e-13	structural molecule activity	2.73e-37
negative regulation of gene expression	1.82e-12	ATP-binding	3.01e-36
negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1.64e-11	ATP	1.10e-23
negative regulation of nitrogen compound metabolic process	1.73e-11	nucleotide binding	5.09e-23
negative regulation of transcription	2.23e-11	kinase	2.32e-18
negative regulation of macromolecule biosynthetic process	4.77e-11	stress response	5.07e-17
mutagenesis site	1.34e-10	purine ribonucleotide binding	9.49e-15
regulation of transcription from RNA polymerase II promoter	1.78e-10	ribonucleotide binding	9.49e-15
ubl conjugation	2.70e-06	purine nucleotide binding	1.38e-13
•		binding site:ATP	1.50e-12
•		nucleotide phosphate-binding region:ATP	3.01e-12
•		phosphotransferase	3.94e-12
•		nuclear lumen	1.74e-10

An interaction network of these kinases in yeast and their direct interaction partners restricted only to direct physical interactions in the nucleus was generated [Figure 34: Nuclear Phosphorylation Network of Yeast]. *IME2*, which is the second most upregulated nuclear kinase upon DR, acts on the induced transcription factors *IME1* and *NDT80* as well as on the suppressed *SUM1*. In *C. elegans*, induced kinases were also enriched among other terms for female meiosis (q-value < 0.03). In line with the enrichment of reproduction-related terms in the DR-essential network in *Drosophila*, induced kinases restricted to the nucleus were enriched for female gamete generation/sexual reproduction/regulation of cell cycle/multicellular organism reproduction (q-value < 5e-3/0.02/0.02/0.02. Finding upregulation of reproduction-related genes is surprising given the known suppressive effect of DR on reproduction (Shanley & Kirkwood, 2000).

–

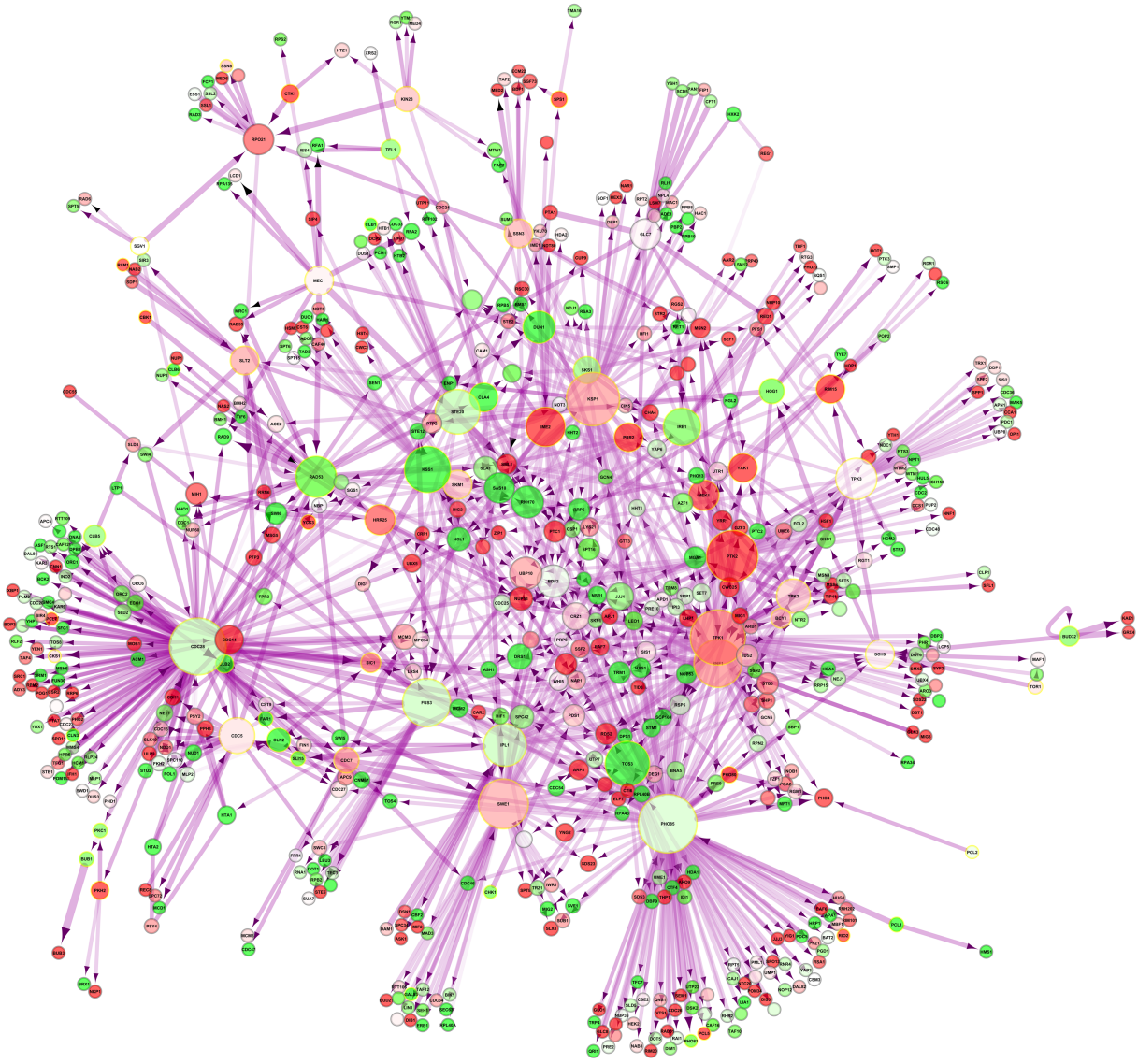

Figure 34: Nuclear Phosphorylation Network of Yeast*. DR-upregulated genes are shown in red and downregulated genes in green. Kinases are marked by a golden halo.*

To extend the approach to mammals, the transcriptional signatures of DR in mammals (Hong, et al., 2010; Swindell, 2008a; Swindell, 2008b; Swindell, 2009), which are about 1348 genes, were utilized and pseudo intensities generated of 2.0 and 0.5 corresponding to genes which are consistently across tissues and experimentally settings up- and downregulated (the default is set to 1.0). Among the terms identified to be consistently significantly (p-value < 0.05) associated to the induced interactions in yeast, worm, fly and

mammals were phosphorylation-related terms and reproductive developmental process [Table 6: Changed Interactions by DR Common to Invertebrates and Mammals].

The DR-essential genes networks in *S. cerevisiae* and *C. elegans* were enriched for sporulation/meiosis and meiosis respectively, although the latter was not significant. Reproduction-associated genes such as *mes-4* are also significant interaction partners of DR-essential genes (total = 17; specific = 4; specificity = 0.24; p-value = 9.87e-05).

In *C. elegans* induced meiotic related kinases were *air-2* (Aurora/Ipl1 Related kinase), *kgb-1* (Kinase, GLH-Binding), *mek-1* (MAP kinase kinase or Erk Kinase), *plk-1* (POLO Kinase), *zen-4* (Zygotic epidermal Enclosure defective).

In fruit fly, reproduction associated induced genes were, for instance *Axn* (Axin), *CDC2*, *cycE* (Cyclin E), *TOP1* (Topoisomerase 1) and *dap* (dacapo) as well as *CDC2* (Cell division control protein 2 homolog), *Ikb1* (DmeI_CG9374), *baz* (bazooka), *koko* (kokopelli), *put* (punt), *sax* (saxophone).

dDnmt2 (MT2), its interaction partner Ipod as well as psq and 3 isoforms of lola were translational upregulated upon DR. Rbf is downregulated on the transcriptional level.

There was even an enrichment for stem cell division (q-value = 7.0e-3) in the DR-induced nuclear kinases of *Drosophila*.

Even mammals induce expression of nuclear kinases which were enriched for response to DNA damage stimulus (q-value = 2.4e-2): *Csnk1e* (casein kinase 1, epsilon), *Cdkn1a* (p21), *Foxo3h*, *Gtf2h1* (p62, which is related to autophagy) and *Msh2* (mutS homolog 2).

DR induces the expression of genes annotated with embryogenesis (23 in mice); however it did not reach significance.

In the mammalian interaction network of DR-differentially expressed genes the most upregulated term was acetylation (p-value = 3.9e-15; q-value = 8.04e-13). Reproductive developmental processes was among the terms of the induced interactions in mammals (p-value = 0.037; q-value = 0.48). Among the upregulated genes in this category were *Cited2*, *Pten*, *Msh2*, *Dld* (dihydrolipoamide dehydrogenase), *Nrip1* (nuclear receptor interacting protein 1) and *Hsd17b4* (hydroxysteroid (17-beta) dehydrogenase 4). To mention there are two DR-induced transcription factors associated to reproductive development: *ZBTB16* and *FOXO3* (a DR-essential ortholog).

4.3.6 Transcription Factors Governing the DR Signatures from Yeast to Mammals

To ascertain the underlying cause of differential expression upon DR gene regulatory networks (transcription factor - target gene interactions (Abdulrehman, et al., 2011; Balaji, et al., 2006)) were utilized to identify candidate transcription factors responsible for the differential expression upon DR. The criteria were, firstly, factors which regulate DR-differentially expressed genes with a high specificity, and secondly, interact with DR-essential genes either at the physical or genetic level.

In yeast, transcription factors which had very high specificity (i.e. specific / total interactions in %) for controlling DR-induced genes were in addition to nutrient-sensing, e.g. Msn2/4 (26%), Gis1 (53%), Mig1 (40%), and stress-responsive transcription factors, such as Hsf1 (34%), which are known to be important for DR-induced lifespan extension, strikingly also meiotic transcription factors, like Ime1 (41%), Ume1 (75%), Ume6 (38%) and Ndt80 (26%).

Sequences (500 bp upstream) of the promoter regions in yeast of the more than two-fold differentially expressed genes were scanned/examined for motif enrichment (hypergeometric test) and indeed upregulated genes are significant enriched for the STRE (stress-response element; p-value = 0.0034/5.67e-4), PDS (post-diauxic shift) element (p-value = 3.89e-7/3.37e-3), URS1 (upstream regulatory sequence; p-value = 1.09e-3), MSE (middle-sporulation element; p-value = 3.85e-3) including the motifs of Ume6 (p = 6.71e-6), Ime1 and Ndt80 (p = 0.013324). Motifs of Ume6, Ime1 and Ndt80 were highly enriched in the promoters of DR-induced genes, while the motif of the repressor of meiosis initiation, Rme1, is significant enriched in the downregulated gene promoters [Table 7: TFBSs Enriched in Yeast DR-Differentially Expressed Genes]. Interestingly, URS1 as well as motifs of Ume6, Sum1, Ndt80 and

Ime1 are even enriched in the upstream sequences of DR-essential genes [Table 8: TFBSs Enriched in Yeast DR-Essential Genes]. Also in another signature of DR (Lin, et al., 2002), meiotic binding motifs were significantly enriched in the 500 bp upstream regions of induced genes. For instance, YGNCACAAAW (*NDT80*) was present in 11 DR-induced genes of the 14 genes in the genome harbouring such a motif (p-value = 0.0025, q-value = 0.029). In this signature Ime1 and Ndt80 had also a high specificity to regulate DR-differentially expressed genes [Figure 37 Transcriptional Regulation of DR in Yeast]. *NDT80* transcript level increase as function of the strength of restriction (Lee & Lee, 2008) and were found to be 2.6-fold elevated in the DNA microarray data generated in this study at 0.5% glucose concentration.

Table 7: TFBSs Enriched in Yeast DR-Differentially Expressed Genes. Meiotic transcription factor binding sites are significantly enriched in the promoters of DR-differentially expressed genes.

Factor	Motif	Targets	#Up	#Down	Up p-value	Down p-value
Ume6	TSGGCGGCTAW	56	25	10	6.71e-6	0.32
URS1	DSGGCGGCND	216	68	31	3.57e-5	0.83
Ime1	TRGSCGSCKA	78	27	13	0.0008	0.42
MSE	HDVKNCACAAAAD	122	35	13	0.0084	0.26
Ndt80	YGNCACAAAA	77	23	10	0.0133	0.76
Rme1	GWACCTCAARA	8	0	5	1	0.00041

Table 8: TFBSs Enriched in Yeast DR-Essential Genes: Meiotic transcription factor binding sites are significantly enriched in the promoters of DR-essential genes.

Factor	Motif	p-value
URS1	GGCGGC	0.0009
Ume6	SGCGGYWV	0.002
Sum1	GYGWCASWAAW	0.009
Ndt80	YGNCACAAAA	0.04
Ime1	TRGSCGSCKA	0.04

In *C. elegans* the p53 homolog CEP-1 is associated to reproduction and its motif (RCWWGYYY) is significant enriched (p-value = 0.048) in the regulatory regions (+800 to -500) of the DR-induced genes (glucose restriction (Lee, et al., 2009), > 1.5-fold-change). The top cluster of terms enriched in the 907 DAF-16/FOXO target genes is multicellular organism reproduction (q-value = 7.9e-5) (Schuster, et al., 2010). Among them is for instance the single lamin gene in *C. elegans lmn-1* that is ubiquitous, expressed in all cells except in cells undergoing spermatogenesis. The 98 progeric genes in *C. elegans* which shorten long-lived *daf-12* mutants lifespan are significantly enriched for larval development (p-value = 4.5e-10; q-value = 8.4e-8), and reproductive developmental process (p-value = 3.3e-9; q-value = 2.5e-7). dFOXO is a positive regulator of sexual reproduction related genes in an Insulin/IGF-like signalling mutant as evidenced by a high enrichment of sexual reproduction associated genes (q-value = 4.7e-2) among those genes that are physically bound by dFOXO, downregulated in short-lived dFOXO-/- mutant and upregulated in long-lived dominant-negative insulin receptor overexpression mutants (Alic, et al., 2011).

Next the mammalian promoter regions of DR-differentially expressed genes were scanned for enrichment of *cis*-regulatory elements and again factors interacting with DR-essential orthologs which are associated to reproductive processes had a high significant enrichment of their motifs in the regulatory sequences of the upregulated genes in the mammalian DR signature [Table 9 TFBSs Enriched in Mammalian DR-Differentially Expressed Genes].

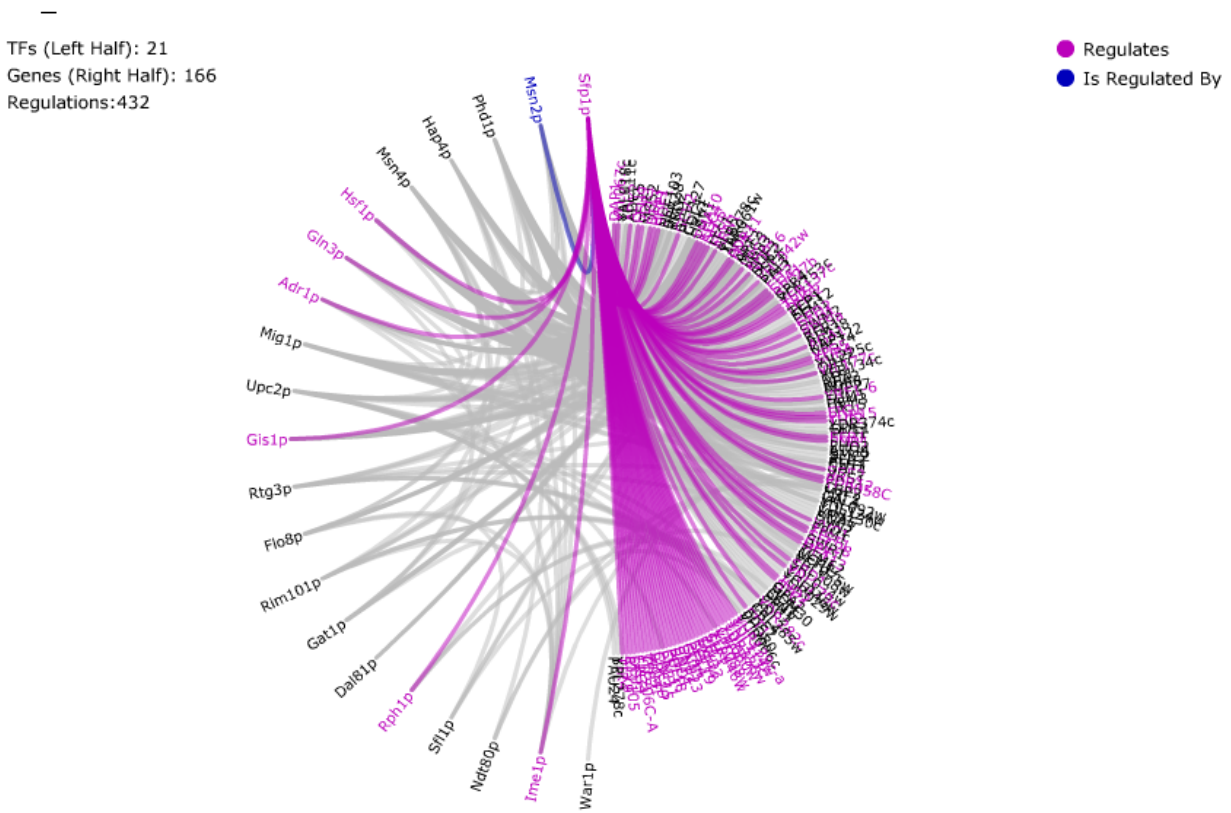

***Figure 35: Transcriptional Regulation of DR in Yeast**: Transcription factors identified via guilt-by-association to regulate DR-differentially expressed genes.*

Table: 9: TFBSs Enriched in Mammalian DR-Differentially Expressed Genes: Reproductive development-related factors are enriched in the promoters of the mammalian DR signature. The binding sites of transcription factors involved in reproductive development are significantly enriched in the promoter regions of DR-upregulated genes in mammals.

Factor	Motif	# All motif	# Delta motif	# Up	# Down	Up p-value	Down p-value	Delta p-value
Foxo3	TTGTTTAC	1182	58	26	23	0.006	0.300	0.005
Zbtb16	TACTGTAC	548	27	14	8	0.007	0.632	0.028
Ppara	AGGTCAWAGGTCA	13	1	1	0	0.012	1	0.071
Nr5a1	YCAAGGYC	6006	268	100	135	0.030	0.178	0.110
FOXA1	TGTTTGC	4271	169	78	77	0.0001	0.211	0.002

The target genes of *Zbtb16* are mostly enriched for reproductive development process (p-value = 1.8e-2) and acetylation (p-value = 1.9e-2): *Msh2*, *Pten*, *Nrip1*, *Atp5a1* (acetylated in liver mitochondria from fasted mice but not from fed mice), *Plekhf1*, *Pura* (transcription factor that activates c-Myc). The only significant target gene of *Ppara* with a perfect match of its consensus sequence is *Narf* (nuclear prelamin A recognition factor), which is interesting as the only nuclear proteins which are prenylated in mammalian cells are prelamin A and B encoded by *LMNA*, a gene associated to heterochromatin organization and premature ageing.

As enrichment for the motifs of reproduction-related transcription factors was found even in the promoter regions of DR-essential genes and lower dS values than expected by chance, it was tested whether this is

a conserved feature of DR-essentiality even in humans. Indeed, DR-essential orthologs were enriched for the motifs of defined factors [Table 10 TFBSs Enriched in Human DR-Essential Gene Orthologs].

Table 10: TFBSs Enriched in Human DR-Essential Gene Orthologs: Reproductive development-related factors are enriched in the promoters of human DR-essential gene orthologs. The binding sites of transcription factors involved in reproductive development are significantly enriched in the promoter regions of mammalian DR-essential gene orthologs.

Factor	Motif	# All motif	# Delta motif	p-value
TP63	RRRCWYGYYY	7652	67	0.003
FOXA1	TGTTTGC	4271	37	0.007
HSF1	ATGGAABD	5807	49	0.008
FOXO3	TTGTTTAC	768	9	0.010
ZBTB16	TACTGTAC	351	4	0.042

TP63 is a DR-essential ortholog and itself regulates DR-essential gene orthologs. *PPARA* and *CITED2* are also direct target genes of p63, whereas PPARA regulates specific *TP63* isoforms (Pozzi, et al., 2009).

–

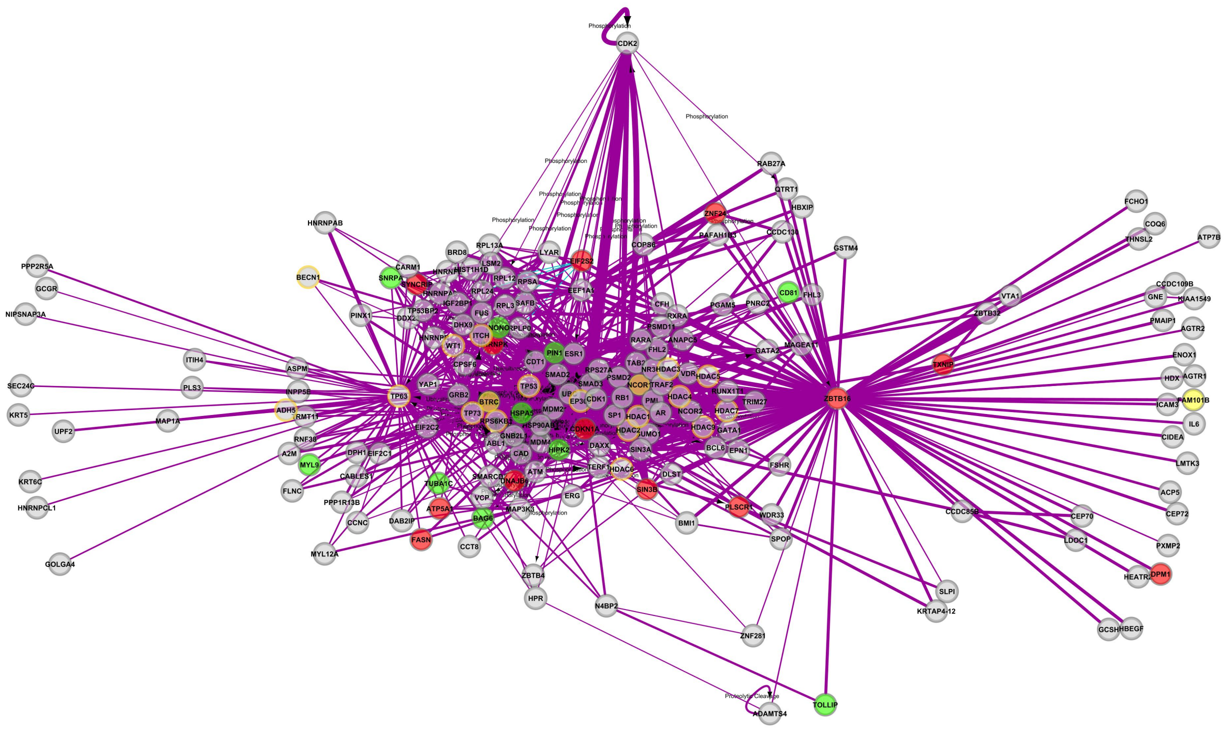

***Figure 36: TP63 and ZBTB16 Interactions**: TP63 and ZBTB16 are significant interaction partners of DR-essential genes and targeted by CDK2/Ime2. Genes up- or downregulated upon DR are in red and green, respectively, while genes which were enriched for up- and downregulation are in yellow. DR-essential orthologs are marked by a golden halo.*

Heat shock factor 1 (*HSF1*) and *FOXO3* are DR-essential orthologs while *ZBTB16* is novel. The mammalian factors identified on the basis of sequence analysis are also significant interaction partners in the networks of DR-essential orthologs in mouse with *Foxo3* (p-value = 7.14e-9), Hsf1 (p-value = 1.41e-7) and *Zbtb16* (p-value = 7.36e-4) as well as in humans with *HSF1* (p-value = 0.017), *ZBTB16* (p-value = 0.020), *TP63* (p-value = 0.041). ZBTB16 and TP63, both are targets of CDK2, the mammalian Ime2 homolog [Figure 36 TP63 and ZBTB16 Interactions].

ZBTB16 is downregulated by *TP63* RNAi, indicating that it is either a direct or indirect target gene of TP63. DR-essential *NNMT1* is right upstream of *ZBTB16* in mouse and humans. *ZBTB16* and *TP63* are coexpressed in stem cells (Majo, et al., 2008). TP63, BMI1 and ZBTB16 mediate proliferation potential of cells via parallel pathways through various gene transcription and silencing programs (Senoo, et al., 2007).

TP63 bound genes are enriched for response to nutrient levels (22 genes of 551; q-value: 3.2e-4) and regeneration (p-value = 1.3e-3) as well as negative regulation of cell differentiation (q-value = 8.1e-3) and reproductive developmental process (p-value = 1.8e-2) (Barton, et al., 2010); (Vigano, et al., 2006). Genes identified to harbour a TP63 consensus sequence within 800 bp of their transcription start sites (TSS) are enriched for reproductive developmental process p-value = 2.7e-2 (*CITED2*, *DDX25*, *CENPI*, *CEP57*, *EIF2B2*, *FOXA1*, *PDE3A* as well as *NOTCH1*), vacuole/lysosomal genes (*ACP2*, *ADRB2*, *CTNS*) and nucleolus. Zbtb16 target genes with a recognition sequence within 800 bp of the TSS are enriched for zinc-finger (q-value = 2.2e-2; p-value = 7.5e-5), RhGAP (p-value = 8.6e-4) and gamete generation / sexual reproduction (p-value = 4.7e-3) with for instance *Oct4*, *Dnmt3l*, *Cctc*, and *Pparg*.

4.3.7 DR-Essential Genes Are Triggered by Lifespan-Extending Spermidine

Comparing different longevity interventions for their commonly regulated processes may allow us to pinpoint the gene expression changes most essential for lifespan extension. Similar to DR, treatment with the polyamine spermidine extends lifespan in multiple model organisms such as yeast, worm, fruit fly, as well as in human cells *in vitro* (Eisenberg, et al., 2009).

It was found that, in yeast (Chattopadhyay, et al., 2009), spermidine treatment causes differential expression of DR-essential genes more often than expected by chance: at a threshold of two-fold change, 15 of the 70 DR-essential genes were among the 727 spermidine-differentially expressed genes (hypergeometric p-value = 0.003). This effect appears to be very specific as there was no enrichment observed among genes induced by spermine, another polyamine, which has not to the current knowledge been shown to affect lifespan. Moreover, DR and spermidine signatures regulate an overlapping set of genes (far more likely than expected by chance): at two-fold change, 323 of 727 spermidine-differentially expressed genes were among the 2560 DR-differentially expressed genes (hypergeometric p-value = 4e-4), whereas genes regulated by DR and spermine did not overlap more than expected by chance at any cut-off (e.g. hypergeometric p-value = 0.69 for two-fold changes).

Comparison of the transcriptional signatures of DR (derived from DNA microarray data of this study here) and spermidine treatment by looking for common functional enrichment terms among genes differentially expressed with DR and spermidine, indicates that both DR- and spermidine-upregulated genes are highly enriched in sexual sporulation (q-value < 1e-3), peroxisome (q-value = 1e-3), mitochondrion (q-value < 0.02) and ubiquitin conjugation pathway (q-value < 1e-3) and downregulated genes are enriched in sterol metabolic process (q-value < 0.05) as well as pentose transmembrane transporter activity (q-value = 0.014). The set of common functional terms enriched by both spermidine and DR treatments (at a 1.5-fold-change threshold) revealed that both induce autophagy, heat response and sporulation. Spermidine treatment leads to both a significant induction and suppression of sporulation/meiosis genes and strikingly similar to DR, induces NDT80 expression.

4.3.8 Proteins and Chromatin Marks Associated to DR-Differentially Genes

DR-differentially expressed genes were scanned in yeast for enrichment and depletion of chromatin-associated proteins and chromatin modifications (Kurdistani, et al., 2004; Pokholok, et al., 2005; Xu & AL, 2005) and it was found that indeed they are enriched and depleted for specific DNA-binding proteins as well as histone acetylation and methylation marks. For DNA-binding proteins their overrepresentation was examined in the two-fold differentially expressed genes with a hypergeometric test. DR- and spermidine-induced genes were most strongly enriched for Ume6 and Sum1, respectively.

Specifically for chromatin marks, the comparisons up versus down and differential versus non-differential (two-fold) was tested (by a binomial test). DR-induced genes were enriched and DR-suppressed genes were depleted for H3, H3K4me1/me2 / K14ac / K14me3/K27ac / K56ac / H4K8ac (p-values: 2.1e-2/2.0e-10/2.9e-4/8.7e-3/1.7e-15/9.6e-10), H2BK11ac/K16ac (p-values: 9.6e-10/5.6e-10) and Gcn4

binding (p-value = 7.3e-4), while it was vice versa for H3K9ac/K18ac/K36me3 (p-values: 5.3e-4/5.03e-7/2.33e-9) and H4K79me3 (p-value = 4.6e-4).

Spermidine treatment, which extends lifespan of multiple species, is also associated with specific chromatin marks on the differentially expressed genes, but it appears to oppose global histone mark changes on the up- vs. downregulated genes by DR in the opposite manner, while enriched or depleted in the same direction by the comparison differential vs. others. For instance, the differentially expressed genes (both up- and downregulated genes) by DR and spermidine are enriched for H3K56ac by 0.012 and 0.022 but greatly depleted on other non-differentially expressed genes by 0.0098 and -0.0026, respectively (binomial p-values: 0.002 and 0.03).

4.4 Discussion

4.4.1 Molecular Conservation of DR-Essential Genes

The findings here show that DR-essential genes are conserved at the molecular level, interact with each other more than expected by chance and therefore allow identification of novel candidates via the guilt-by-association principle.

DR-essential genes interact with phosphorylation-related and ageing genes. Nutrient-sensing signalling is intrinsically coupled to growth regulation and a link between growth and DR signalling pathways is well-established (de Magalhaes, et al., 2012). Some growth programs are differentially important in various organisms that may be reflected in other effectors downstream DR in different species. In addition, terms associated with translation were suppressed in the comparison of the interactomes of multiple species [Table 5: Changed Interactions by DR Common to Invertebrates], again in line with previous findings (de Magalhaes, et al., 2012).

DR may be considered to act as a nutritional stressor. Gluconeogenesis genes located at subtelomeric regions are known to be upregulated by stress (Mak, et al., 2009) via evolutionarily conserved epigenetic control (Smith, et al., 2011). In fact, key enzymes and factors of gluconeogenesis are induced by DR which may sequester TORC1 (Brown, et al., 2010) away from its normal functioning membranes (i.e. vacuolar and endosome membrane, etc.).

Rpd3 deacetylation mediates Ume6 transcriptional repression activity (Rundlett, et al., 1998), which is converted to an activator by Ime1 binding and Ime2 phosphorylation. DR-essential *HSF1* is already known to be involved in gametogenesis in mammals and even to be essential for meiosis (Akerfelt, et al., 2010; Bierkamp, et al., 2010; Le Masson, et al., 2011; Metchat, et al., 2009). Inducible HSF1-binding sites are associated with histone acetylation (Guertin & Lis, 2010).

The finding that DR-essential genes are molecular conserved reflects the observation that DR extends lifespan in various evolutionary distant related organisms (yeast, worm, fly, spider, fish, dog, hamster, mouse, rats, monkeys, etc.).

Via the guilt-by-association concept novel DR-essential genes were identified primarily implicated in drug detoxification, transition metal ion homeostasis and vacuolar trafficking / endocytosis, as well as a potential role of S-adenosylhomocysteinase as a common interactor of DR-essential genes in yeast, worm, fly and mammals. Several DR-essential genes change their expression level upon DR, some even across species, such as genes encoding the S-adenosylmethionine synthetase activity. DR-differentially expressed genes are associated with specific chromatin marks on AL, some are even shared with lifespan extending spermidine treatment.

Here it was found that S-adenosylmethionine synthetase is commonly differentially expressed upon DR, while S-adenosylhomocysteinase is commonly interacting specifically with DR-essential genes in multiple organisms.

Kinase signalling is dramatically suppressed in very long-lived PI3K mutant worms, and S-adenosylhomocysteinase, several insulin-like peptides, and DAF-15 all turn up in proteomics and phosphoproteomic studies.

A common theme of longevity mutants is the silencing of multiple signalling pathways, which is also evident in an attenuation of total kinase activity resulting in proteome-wide reduced phosphorylation in, for instance, strong alleles of *age-1* mutants (Shmookler Reis, et al., 2009).

It is clear that diet alters epigenetic marks (i.e. chemical tags such as acetyl or methyl groups) and results in an altered chromatin state. Stress alters the methylation state of histones in fruit fly with transgenerational inheritable effects (Seong, et al., 2011). The lifespan prolonging effect of DR is inherited to the next generation in rotifer (Kaneko, et al., 2010). Deficiencies in H3K4 trimethylase complex components ASH-2, WDR-5 or SET-2 in parental generation extend lifespan of descendants up until the third generation (Greer, et al., 2011). Moreover, diet has also been shown to alter gene expression transgenerationally in mammalian species (Flintoft, 2011; Ng, et al., 2010). In humans the rate of mortality is affected in a transgenerational manner by food intake in the parental generation (Kaati, et al., 2007). Food intake in one generation influences genes expression (Benyshek, et al., 2006), glucose and cholesterol metabolism (Benyshek, et al., 2006; Carone, et al., 2010) and β-cell function (Ng, et al., 2010) in the subsequent generation in mammals. Thus, dietary intake affects chromatin marks that may not be completely erased between generations. The lifespan prolonging effect of DR can be mimicked or blocked by α-lipoic acid supplementation during AL, which exerts a memory effect of the previous feeding regimen in feeding switch experiments (Merry, et al., 2008) and is associated with an altered proteome acetylation state, with a hyperacetylated state linked to longevity. Taken together this evidence indicates that an altered chromatin state in DR is its principal major lifespan extending mechanism across species boundaries, but why and how?

4.4.2 Differential Expressed DR-essential Genes

Numerous DR-essential genes and their orthologs exhibit the expected change already either on the transcriptional or translational level. However, this was not observed at the genome-scale at all cutoffs. For commonly used cutoffs, DR-essential genes are not more likely to be differentially expressed than expected by chance. The problem here may be that in yeast there are at least 3 different longevity definitions used in the literature and at least as many different versions of DR. If one focused on just one, say replicative lifespan (the bud counting assay), and moderate glucose withdrawal (0.5%), a significant enrichment might be seen. The microarray data showed that numerous DR-essential genes are differentially regulated by DR. Moreover, other longevity manipulations such as spermidine treatment also differentially regulate DR-essential genes. Thus the systems biology analysis here can be expanded to other organisms and be predicted that longevity might converge on influencing intracellular polyamine levels.

4.4.3 PI3K

PI3K activity positively regulates organismal reproduction and developmental progression. However, at the cellular level it inhibits the expression of reproductive (i.e. germline) genes in the somatic cells. Thus ectopic downregulation of PI3K ceases organismal reproduction, but induces germline-trait-like expression in the soma. Fascinating, PI3K tightly regulates gametogenesis in mammals.

4.4.4 FOXOs Regulate Entry Into Gametogenesis

FoxOs promote organismal longevity in invertebrates, and in humans single nucleotide polymorphisms in FOXO3 are associated with extreme longevity (Kenyon, 2010).

Foxo1 mediates some aspects of the DR effect (Yamaza, et al., 2010). PI3K signalling regulates stability and subcellular localization of Foxos, including Foxo1 (Goertz, et al., 2011). FoxOs control key aspects in stem cell maintenance as they regulate self-renewal in hematopoietic and neural stem cells (Paik, et al., 2007; Tothova, et al., 2007; Renault, et al., 2009). FoxOs, particular Foxo3 co-ordinately regulates neural stem cell homeostasis through genes influencing stress response and oxygen metabolism (Renault, et al., 2009; Paik, et al., 2007). FoxOs regulate hematopoietic stem cell differentiation and assist long-term maintenance by protecting against oxidative stress.

FoxO function in male and female germ-line shares some similarity. FoxOs evolved to control gametogenesis within the gonad itself. Whereas Foxo1 is highly expressed in undifferentiated

spermatogonia, Foxo3 is highly expressed in primordial oocytes, in which it serves to retrain their growth (John, et al., 2008). Foxo3 function in female germ line maintenance (Castrillon, et al., 2003; John, et al., 2007; John, et al., 2008; Brenkman & Burgering, 2003), while Foxo1 controls multiple steps of spermatogenesis, from SSC proliferation and self-renewal to progression of spermatogenesis, including meiosis. Spermatogonial stem cell (SSC) are capable of self-renewal and immortal growth. Foxo1 specifically marks mouse gonocytes and a spermatogonia subset with stem cell potential. Foxo1 was required for both spermatogonial stem cells homeostasis and initiation of spermatogenesis. Combined deficiency of Foxo1, Foxo3, and Foxo4 resulted in a severe impairment of SSC self-renewal and a complete block of differentiation, indicating that Foxo3 and Foxo4 contribute to SSC function. PI3K controls both SSC maintenance and differentiation. The PI3K-Akt pathway is the principal pathway regulating Foxo1 subcellular localization and activity in the context of spermatogenesis. FoxOs appear to control a network of genes unique to spermatogenesis. FoxOs join the growing network of transcription factors - including Zbtb16 and Taf4b - that control early steps of spermatogenesis, including SSC self-renewal (Buaas, et al., 2004; Falender, et al., 2005).

Forkhead transcription factors control crucial steps in embryogenesis and are essential for the development of all germ layers and organs (Lehmann, et al., 2003). Foxo3-/- mice exhibit signs of accelerated ageing and have global follicular activation, therefore become prematurely infertile too (Castrillon, et al., 2003). Primordial follicles (which are in pre-meiotic phase) might be regarded as being in a state of developmental arrest. To maintain this arrest, FoxO3a is apparently necessary. Forced FoxO activation induces also an reversible arrest in *C. elegans*, *Drosophila* (Kramer, et al., 2003) and in mammalian cells (Kops, et al., 2002). In general FoxOs are capable of causing a reversible arrest. Stem cells are an example of reversible arrested cells that can re-enter proliferation and/or differentiation. FoxO-regulated genes are required for the maintenance of satellite cells, which include p27Kip1 and p130/Rb2, both of which are also required for muscle differentiation (Bois & Grosveld, 2003).

The unique genetic requirement for Foxo1 in males and Foxo3 in females mirrors their high expression at discrete cellular stages in spermatogenesis or oogenesis.

FoxOs together with ZBTB16 control early steps of spermatogenesis. Foxo1 and ZBTB16 were always coexpressed during spermatogenesis, where their expression was restricted to undifferentiated spermatogonia. ZBTB16 indirectly regulates mTOR activity (Fraser, et al., 2002).

Foxo1 transcriptome in male germ cells was distinct from that in other cell types, for which genes regulating oxidative stress resistance were rendered as major targets (Tothova, et al., 2007; Renault, et al., 2009; Paik, et al., 2009).

GnRH (gonadotropin-releasing hormone) regulates FOXOs. FOXOs regulate LHB subunit expression. Foxo targets GADD45, FasL, p21Cip1 and p27Kip1. Foxo3a mediates GADD45 and FasL expression in response to GnRH. GnRH is an essential regulator of the reproductive processes by stimulating the synthesis of LH (Luteinizing Hormone) and FSH (Follicle-Stimulating Hormone) in pituitary gonadotropes, thereby regulating gametogenesis and steroidogenesis (Stavrou, 2011).

4.4.5 ZBTB16

Loss-of-function mutations in ZBTB16 also called promyelocytic leukemia zinc finger protein (PLZF) cause an age-dependent loss of germ-line stem cells leading to sterility. *ZBTB16* is expressed in germ-line stem cells and is required for their self-renewal. *ZBTB16* facilitates transcriptional repression through recruitment of chromatin modifying enzymes to specific DNA sequences.

4.4.6 eor-1

Blasting ZBTB16 identified *blmp-1* as the closest sequence homolog, which directly regulates *cep-1*/p63. However, *eor-1* is the *C. elegans* ortholog of mammalian ZBTB16 (Zhang, et al., 1999). ZBTB16 does influence developmental patterning and Hox gene expression in mouse (Barna, et al., 2000).

Epidermal growth factor (EGF) promotes cell division and cellular differentiation in developing animals. EGF acts through phospholipase Cy and IP3 receptor signalling maintains pharyngeal and body wall muscle formation in ageing adults and delays accumulation of lipofuscin-enriched ageing pigments within

intestinal cells. EGF also acts through RAS/ERK pathway to regulate protein homeostasis by promoting antioxidants gene expression, stimulating activity of the ubiquitin proteasome system (UPS) and repressing expression of small heat shock protein chaperones. Effects of EGF signalling on lifespan are largely independent of IIS, as effect of, EGF signalling mutants on lifespan are largely independent of DAF-2 and DAF-16 mutants. However, these two signalling pathways have multiple points of overlap, coordination and cross talk. IIS and EGF signalling respond to environment and to developmental timing, respectively. *let-23* loss-of-function mutant had a decreased lifespan when raised at 20 degree Celsius. *eor-1* is oocyte enriched. *eor-1* and *eor-2* act downstream or in parallel to SynMuv/Rb pathway to promote RAS/ERK-dependent transcription. EOR-1 interacts with SynMuv B pathway component LIN-36, a possible histone deacetylase complex component (Thomas & Horvitz, 1999; Walhout, et al., 2000; Howard & Sundaram, 2002). SynMuv gene products could antagonize Ras signalling in part by binding to and inhibiting activity of positively acting transcriptional regulators such as EOR-1. LIN-36 EOR-1 interaction could also be involved in positive role of some SynMuv N genes (Chen & Han, 2001). *eor-1* regulates cooperatively Hox-dependent patterning (Howard & Sundaram, 2002).

4.4.7 Transcription Factor Mediated Rejuvenation Triggered by Dietary Restriction

TP63 overexpression increases ROS levels and ROS sensitivity (Ellisen, et al., 2002). The optimal consensus motif of p63 consists of a C(T)TG core and an AT-rich 5' and 3' flanking sequence (Ortt & Sinha, 2006), which differentiates it from its paralog p53. The consensus motif gene that are regulated by p63 were identified (Perez, et al., 2007). p53 gene family target genes were predicated from gene expression profiles of mutants and pattern matching (Sbisa, et al., 2007). The function of its homolog TP53 in ageing is controversial. Moderate overexpression of p53 in fruit fly can lead to lifespan extension (Hur & Walker, 2009), while Dmp53-null mutants develop into adults, but display mild defects in longevity and reproduction (Lee, et al., 2003). In fruit fly the process of meiotic recombination instigates programmed activation of p53 in the germ line (Lu, et al., 2010b). Fly p53 regulates neural stem cell self-renewal (Ouyang, et al., 2011). Expression of short and long p53 isoforms may maintain a balance between tumor suppression and tissue regulation (Maier, et al., 2004).

4.4.8 Characterization of DR-Essential Genes

Here a class of genes that is essential for the lifespan extending effect of dietary restriction was defined and members in different species compiled and characterized at the level of molecular evolution and molecular interactions. Those DR-essential genes were found to be more conserved than expected by chance and more likely to interact with each other, indicating that they are evolutionary conserved and acting together in a network. Based on this assumption, a guilt-by-association method utilizing the interactome was used to predict novel DR-essential genes in multiple model organisms and humans by performing orthologous complementation. Top candidate genes that were further restricted by filtering for processes and location associated with DR-differentially expressed genes (autophagy and lysosome/vacuole) were tested in lifespan experiments (by Fusheng Tang). 8 of 9 mutants were found to affect lifespan and several were DR-essential. Further mutant cells tend to exhibit changed vacuoles. DR-essential genes were found to be more likely to be differential expressed under DR than expected by chance. And the effect of spermidine, which is assumed to be a DR mimetic that induces autophagy just like DR were found to differentially express DR-essential genes far more likely than expected by pure chance. Interaction networks were integrated with transcriptomics data of DR and commonly stimulated and suppressed interactions identified via comparative approach across species. Potential transcription factors governing the DR-triggered gene expression changes were identified.

4.4.9 A Database and Analysis of Genes Essential for DR Life-Extending Effects

Understanding the genetic basis of DR is of great importance not only to help elucidate mechanisms of ageing but also to understand how diet can influence ageing, longevity, health, and age-related diseases, which may have major human therapeutic applications (Bishop & Guarente, 2007; de Magalhaes, et al., 2012). DR-essential genes are no doubt crucial pieces of the puzzle to solve the mechanism of DR (Bishop & Guarente, 2007; Fontana, et al., 2010; Gems, et al., 2002), but to date they have not been studied as a unified system. To address the need for a more systematic study of the mechanisms of DR-conferred lifespan extension, GenDR was created, the first database of DR-essential genes and indeed the first database of DR-related genes.

GenDR provides manually curated information on genetic mutations which interfere with the pro-longevity effect of DR as well as their respective homologous genes in the major model organisms and humans. Because different genes appear to be important in different regimens and there are conflicting findings regarding some of them, different DR regimens across multiple model organisms are covered, allowing researchers to focus on their preferred system. With the growing importance of network biology, functional genomics and systems biology to study ageing and DR (de Magalhaes, et al., 2012), GenDR will be a valuable tool for researchers to study the genetic and molecular mechanism of DR as evidenced by the validation of vacuole mutants [Figure 27: DR Vacuole-Associated Genes Are Required for DR Lifespan Extension and Normal Vacuolar Morphology] and gene regulatory inference.

The study of the molecular evolution of DR-essential genes [Figure 22 DR Genes Are Molecular Conserved and Form a Tight Network] showed conservation at the molecular level reflecting the observation that DR extends lifespan in various evolutionarily distant organisms. Interestingly, DR-essential genes had lower dN/dS ratios than expected purely by chance, as well as lower dN and dS values. This could indicate that natural selection has constrained not only the amino acid sequences, but also the nucleotide sequences which may include regulatory elements. The molecular interaction data support the observation that DR-essential genes are conserved across vast evolutionary distances, as genes with relatively high degree, i.e. hubs, are more ancient and evolve more slowly than genes that encode non-hub proteins (Fraser, et al., 2002; Eisenberg & Levanon, 2003; Saeed & Deane, 2006). One caveat of this analysis is that these results may also reflect a bias in the selection of genes, as researchers tend to study genes which have orthologs and have been shown to affect DR in other organisms. Similarly, the result that DR-essential genes interact with each other more than expected by chance might also be affected by selection bias, as genes studied in the context of DR are often part of similar or closely interacting biological pathways (e.g. insulin/IGF1 signalling and TOR). Also, both DR mechanism and ageing-effect genes tend to be enriched for signal transduction genes, especially those which are well known to be highly conserved (Kim, 2007).

4.4.10 Network Analyses Reveal Novel DR-Essential Genes and Candidate Mechanisms

The network analyses here successfully predicted novel DR-essential genes in yeast. This illustrates that it is possible to associate *in silico* genes that are of interest in a DR context, with other, little-known genes, and derive testable new hypotheses. Network analyses based on GenDR have therefore the capacity to implicate new genes related to lifespan extension by DR. In particular, eight novel vacuole-related DR-essential yeast genes that impair DR-mediated longevity when deleted were identified, of which three (*OPT2*, *FRE6*, and *RCR2*) extended lifespan in AL and prevented any further lifespan extension by DR. Although the genome-wide frequency of DR-essential genes is unknown, finding 8/9 of such genes with 3/9 extending lifespan in AL is likely much more than expected by chance and illustrates the utility of the here employed method.

These genes had been previously implicated in drug detoxification (*OPT2*), transition metal ion homeostasis (*FRE6*) and endosomal-vacuolar trafficking of plasma membrane proteins (*RCR2*, *VPS20*, *GTR1*, *SLM4*) as well as a vacuolar membrane protein *DAP2*, plus a protein of unknown function (*YDL180W*). Comparing localizations of the proteins encoded by these genes with the known DR-essential genes in the database revealed endocytosis as one target of DR [Figure 7 Localization of Vacuole-Related

DR Proteins & Lipids in Yeast Cells]. Moreover, the different effects on lifespan of mutants missing these newly identified DR-essential genes [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes] prompted the speculation on the function of each endocytic step in longevity.

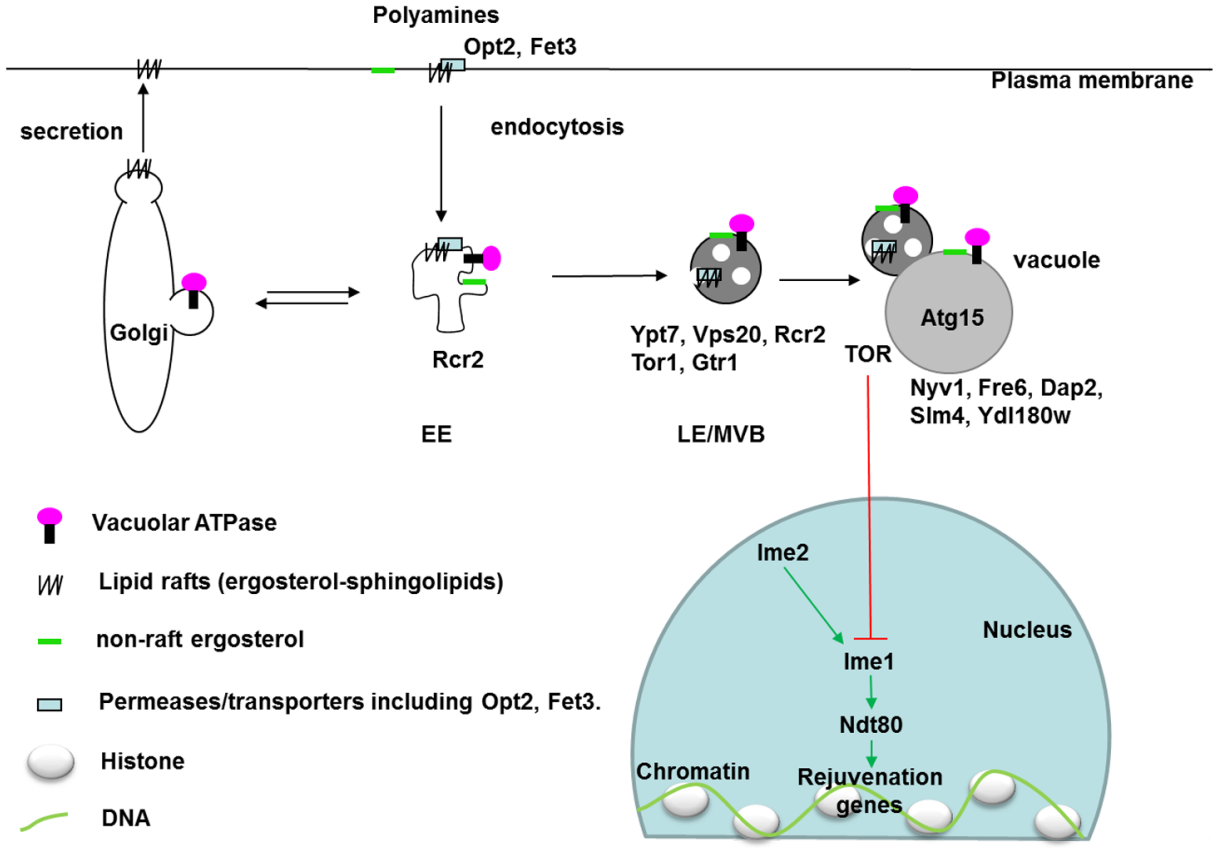

Figure 37: Localization of Vacuole-Related DR-Essential Proteins and Lipids in Yeast Cells. *In response to environmental triggers such as DR, membrane transporters (Opt2 and others) on the plasma membrane are endocytosed through lipid rafts (ergosterol/sphingolipids) after ubiquitinylation. On early endosomes, proteins and lipids are sorted to late endosomes, Golgi, or other organelles. In late endosomes, different endosomal sorting complexes required for transport (ESCRT) push the target proteins with their membranes into the lumen of late endosome, or multivesicular bodies (MVB). Matured MVB can fuse with vacuoles, within which proteases and lipases (Atg15) degrade target proteins and lipids. In mammalian cells, maturation of late endosomes is essential for activation of the master regulator for growth, the mammalian target of rapamycin 1 (mTORC1). In yeast cells, TORC1 suppresses meiotic transcription factors which are normally only active during gametogenesis (Colomina+Al:2003). The inhibitory effects of DR on TORC1 thus activate these rejuvenation genes. EE: early endosome. LE: late endosome. MVB: multivesicular body. Black arrows indicate movement of vesicles. DR-essential proteins are labelled in their localized organelles. Localization data were extracted from the SGD database. Ypt7 localization on late endosomes was reported by (Balderhaar+Al:2010). Green arrows indicate activation and a red line indicates an inhibitory effect.*

Cells take up nutrients through plasma membrane transporters including oligopeptidase transporter Opt2 and ferrous transporter Fet3. Secretion and endocytosis determine the fate of these transporters depending on environmental cues [Table 5 Changed Interactions by DR Common to Invertebrates]. Both secretion and endocytosis depend on the lipid composition of membranes. Blocking ergosterol synthesis (erg6Δ) halts the degradation of Tat2, the high-affinity transporter of tryptophan, and traps it in late endosomes (or multivesicular bodies) (Umebayashi & Nakano, 2003). Nutrient transporters destined for degradation in vacuoles are ubiquitinated by Rsp5 (a DR-essential ortholog of *C. elegans* WWP-1 (Carrano, et al., 2009)) on the plasma membrane or endosomes (Nikko & Pelham, 2009). Ubiquitinated cargoes are sorted to late endosomes, where endosomal sorting complexes required for transport

(ESCRT) work sequentially to encapsulate the ubiquitinated transporters within the endosomal lumen. Vps20 is a component of ESCRT III, which seals the membranes (Babst, 2011). The identification of *VPS20* as a DR-essential gene may suggest the involvement of late endosomes in longevity. In support of this possibility, a screen for mutants that shorten yeast chronological lifespan also revealed a critical role of the late endosome (Fabrizio, et al., 2010; Longo, et al., 2010).

For several of the mutants tested the lifespan was shorter than wild-type, which indicates that these genes are required for normal longevity and especially for the response to DR (perhaps due to their involvement in endocytosis). There are also four mutants (opt2Δ, rcr2Δ, vps20Δ, fre6Δ) with a shorter lifespan on DR than on AL [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes], comparable to the "inverse DR" effect reported for erg6Δ, ypt7Δ, and nyv1Δ mutants (Tang, et al., 2008). These mutants are all likely to be blocked in the maturation of late endosomes. Deletion of Rcr2 or blockage of ergosterol synthesis (erg6Δ) led to accumulation of endocytic vesicles (or a special subset of apparent vacuoles as visualized by the dye FM4-64) that are unable to fuse with other vacuoles, and to the apparent formation of highly fragmented vacuoles [Figure 27: DR Vacuole-Associated Genes Are Required for DR Lifespan Extension and Normal Vacuolar Morphology B]; also see Figure 4B of (Tang, et al., 2008)). The fact that deletion of the plasma membrane transporter *OPT2* also results in fragmented vacuoles (Aouida, et al., 2009) may indicate improper fusion between late endosome and vacuoles. The maturation of late endosomes also depends on the proton gradient across the membrane, which is maintained by a membrane H(+)-ATPase. *FRE6* deletion could impair metal-ion efflux and consequently decrease cytosolic iron and copper levels (Kakinuma, et al., 1981). Copper ions control the activity of membrane H(+)-ATPase (Kakinuma, et al., 1981). As such, endocytosis appears to be essential for normal lifespan under AL and especially for the lifespan extension response under DR conditions. Endocytosis may for instance remove damaged plasma membrane proteins, and in higher organisms even intercellular debris.

In addition to implicating the plasma membrane/endosome/vacuole pathway as one target of DR, the longevity of three mutants (opt2Δ, fre6Δ, and rcr2Δ) under AL [Table 4: Lifespan and Vacuolar Changes of Novel DR-Essential Genes] reveals a putative novel controlling mechanism of the target of rapamycin complex 1 (TORC1). *OPT2* and *FRE6* exhibit negative genetic interactions with *TOR1* (Chan, et al., 2000) and *TCO89* (Costanzo, et al., 2010), respectively, both of which encode components of TORC1. A negative genetic interaction usually suggests a cooperative relationship (Costanzo, et al., 2010). Rcr2 interacts with Ypt7 in a yeast 2-hybrid assay (Ito, et al., 2001). While it is not known to be true in yeast, the mammalian ortholog of Ypt7, Rab7, is essential for mammalian TORC1 activation (Flinn, et al., 2010). It is thus tantalizing to speculate that TORC1 may be partially inactivated in opt2Δ, fre6Δ, or rcr2Δ strains. Considering that part of TORC1 is on the vacuolar and endosomal membranes (Sturgill, et al., 2008), it is not surprising to observe that some vacuolar membrane mutants affect the activity of TORC1. Another beneficial effect of opt2Δ in longevity might be the uptake of life-extending polyamines (see also (Eldakak, et al., 2010)).

4.4.11 Transcriptional Changes upon DR and Their Regulators

There was only a slight enrichment of DR-essential genes among those differentially expressed during DR. This is not unexpected, since DR-essential genes are likely to lie upstream in the DR longevity pathway, whereas most genes expressed differentially upon DR would tend to lie downstream in an effector cascade. It is also possible that many DR-essential genes mediate their effects on lifespan via transient expression, changes in protein levels, or in biochemical activities that are not reflected in analyses of mRNA levels. The fact that it was not differentiated between the various DR regimens would also dilute the enrichment of essential genes shared by multiple pathways. Nonetheless, numerous DR-essential genes show strong changes at the transcriptional and/or translational level, which may correspond to a respective change in the activity level, or might represent a compensatory response to changes in regulation at other levels. For instance, the only validated DR-essential gene in mammals *Ghr*, extends lifespan if deleted and is also found to be downregulated upon DR (Plank, et al., 2012).

Among the DR-essential homologs commonly differentially expressed in all examined species were fatty acid elongases, which extend lifespan when downregulated in *C. elegans* (Shmookler Reis, et al., 2011). In addition, DR regulates S-adenosylmethionine synthetase activity at the transcriptional/translational level in multiple species; its product, S-adenosylmethionine (SAM), is the methyl donor for most methylation reactions. A common significant interactor of DR-essential genes was S-adenosylhomocysteinase

catalysing the reversible hydrolysis of S-adenosylhomocysteine (SAH), which in turn is a competitive inhibitor of methyltransferases. SAM and SAH levels, acting via DR-essential *NNT1*/*NNMT* encoding nicotinamide-N-methyltransferase (Anderson, et al., 2003), would impact NAD+ metabolism and consequently NAD(+)-dependent protein deacetylases (sirtuins) which were also common interactors of DR-essential genes. SAM and SAH levels might also exert pervasive effects via the control of DR-essential ergosterol biosynthesis in yeast, sterol modifications in *C. elegans* (influencing dauer formation), DNA methylation in mammals, as well as histone methylation and polyamine synthesis in all species.

It is clear that diet alters epigenetic marks, resulting in an altered chromatin state that affects gene expression (Jaenisch & Bird, 2003; Burdge, et al., 2007). The study here provides evidence pointing to a fundamental role of chromatin in DR. Chromatin organization was among the most enriched categories of DR-essential orthologous networks in mammals, and most strikingly several terms related to chromatin were enriched among DR-stimulated interactions in the interactomes in multiple species [Table 5 Changed Interactions by DR Common to Invertebrates]. Modified chromatin structure has been proposed as an underlying mediator of ageing from yeast to humans (Feser & Tyler, 2011), and is known to be influenced by diet (Vaquero & Reinberg, 2009). Taken together, the evidence implies that an altered chromatin state in DR is a major component of its lifespan-extending mechanism, conserved across diverse species.

Clearly DR works via a conserved mechanism, but how does it interfere with the ageing process? A phenomenon that is at least equally ancient and conserved is the intergenerational/meiotic reversal of all age-related changes that had accumulated, and the restoration of lifespan for progeny (Unal, et al., 2011). Yeast rejuvenation occurs during sporulation (the equivalent of the mammalian gametogenesis) (Unal, et al., 2011), when transient activation of Ime1 or Ndt80 rejuvenates cells and resets their age to “zero” (Unal, et al., 2011). Here it was found that both Ime1 (1.4-fold) and Ndt80 (2.7-fold) are induced upon DR, leading to the speculation that DR may act on ageing via a mild, continuous form of “rejuvenation”. This would explain why switching the feeding regimen leads to a change in the state, rather than the rate, of ageing – i.e. it lowers the mortality level almost immediately (Mair, et al., 2003).

It was proposed that polyamine biosynthesis may be essential for meiosis and sporulation (Brawley & Ferro, 1979). Spermidine and/or spermine are, in fact, required for sporulation (Tabor, 1981) and transition into meiosis (Tabor, 1981). Polyamine concentration declines with age in yeast and human cells. Supplementation with spermidine increased lifespan in yeast, worms, flies, and human cells, and a polyamine-rich diet (as well as probiotics that induce polyamine production) decreased mortality in mice (Matsumoto, et al., 2011; Soda, et al., 2009). Spermidine treatment results in hypoacetylation of H3K9, H3K14, and H3K18, probably via inactivation of histone acetyltransferases. Inactivation of the responsible histone acetyltransferases extended replicative lifespan and suppressed lifespan extension by spermidine. While spermidine treatment leads to global histone hypoacetylation, histones on specific promoters such as those of autophagy-related genes were actually hyperacetylated and induced in expression (Eisenberg, et al., 2009). Increasing acetylation levels of H4K5 and H4K12 via deletion of DR-essential protein deacetylase *RPD3* also increased lifespan in yeast and *Drosophila*. Glucose and nitrogen regulate the switch from histone deacetylation to acetylation of early meiosis genes (Pnueli, et al., 2004). It is interesting to note that spermidine could only effectively extend replicative lifespan in yeast when treatment was initiated at old ages (Eisenberg, et al., 2009), indicating that it works via a single round of light rejuvenation. Strikingly, genes differentially expressed by DR and spermidine treatment have certain chromatin marks in common, including histone acetylations, while on the global scale they lead to opposing effects (data not shown). As such, it appears that spermidine might be a downstream component of the DR-mediated lifespan extension and chromatin remodelling induced by DR awakens meiotic rejuvenation programs. Intriguingly, induction of interactions between genes annotated with reproduction-related terms was the key shared feature of the comparison of DR interactomes from yeast, worms and flies.

Given the multitude of changes induced by DR, the study of DR-essential genes and their networks is an important new tool to distinguish those genes and processes that are the “drivers” of the life-extending effects of DR from those that are mere “passengers”. The GenDR database will therefore be valuable for numerous researchers and this work demonstrates that DR is suited for a network-guided approach. As anticipated, DR-essential genes are conserved at the molecular level and interact with each other more than expected by chance. Crucially, this work demonstrates how gene network analyses of DR allow the

identification of novel candidates that can be tested experimentally. These results also indicate important roles for genes involved in endosomal/vacuolar trafficking in DR lifespan extension and provide new intriguing candidates for further studies in yeast such as *OPT2*, *FRE6*, and *RCR2*. Genome-wide expression data provide further clues of which genes and processes are regulated by DR, and their systematic integration in interaction networks was initiated in this study. Moreover, gene-regulatory relationships and sequence-based inference both point to an important role of meiotic transcription factors in DR. Finally, by comparing the effects of DR on the interactomes of multiple species, it was discovered that DR suppresses translation while stimulating chromatin reorganization and a defined meiosis-related rejuvenation process, across phyla separated by a billion years of evolution. Many questions still remain. What is the nature of the rejuvenation processes utilized by DR to extend lifespan? What is the exact role of polyamines in DR-induced rejuvenation? Are intracellular polyamine levels increased upon DR? And what factors correspond to the mammalian counterpart(s) of *NDT80*? It is hoped that the efforts in establishing GenDR will help to unravel the secrets of DR-conferred lifespan extension.

4.5 Contribution

Daniel Wuttke did the computational part and Fusheng Tang (Ph.D.; Associated Professor of Applied Science; University of Arkansas at Little Rock, USA) did the experimental part. Co-authors in (Wuttke, et al., 2012) revised the text written by Daniel Wuttke.

5 Dietary Restriction Signatures

Abstract: Dietary restriction is the most powerful non-genetic intervention known to retard the basic ageing process. Understanding how DR interferes with the ageing process would be useful for understanding ageing and developing alternative interventions. Dietary restriction gene expression profiles in human and common biomedical model organisms were utilized to generate molecular signatures (list of genes with differential expression metrics) representing the effect of dietary restriction on the level of gene expression and to identify the associated genes and functional categories enriched in the signatures. DR was found to commonly differentially regulate the circadian clock, stress response, apoptosis and proteolysis. DR specifically upregulates genes involved in chromatin silencing and downregulates inflammation.

5.1 Background

Dietary restriction (DR) reprograms the transcriptome, epigenome and metabolome in a way that slows down ageing [Molecular Profiling to Decipher Ageing]. Although it is known that the intervention increases lifespan from yeast to primates [Dietary Restriction, Dietary Restriction Genes] the exact mechanism is not understood. Physiological responses to dietary restriction are cell-type specific and variable throughout the lifespan (Schafer, et al., 2015). Many studies in multiple types of tissues and varied regimens have performed transcriptionally profiling. Several attempts on establishing consensus signatures of dietary restriction have been made previously Molecular Profiling to Decipher Ageing]. Analysis of previous studies on dietary restriction in mouse identified gene expression changes that are shared among multiple tissues (Swindell, 2009). A common response to dietary restriction in diverse tissues and species was sought to be identified. Thereby gene expression data from eight tissues of mice subjected to dietary restriction was analysed. From this a common transcription signature was identified that includes functional categories of mitochondrial energy metabolism, inflammation and ribosomal structure. Such signature was detected in flies, rats and monkeys on dietary restriction suggesting to be aspects of DR that are evolutionary conserved (Barger, et al., 2015). Meta-analysis of microarray dietary restriction studies in mammals found genes and processes that are robustly altered such as growth hormone signalling, lipid metabolism, and immune response (Plank, et al., 2012).

Whole-genome transcriptional profiling of DR in seven mouse strain in white adipose tissue, gastrocnemius muscle, heart and brain neocortex lead to the the identification of tissue-specific genes that change in expression in multiple strains with DR. Subset of those genes could be used to validate potential pharmacological DR-mimentics (Barger, et al., 2017). Publically available microarray data was used to analyse the expression responses to DR in males from seven mouse strains and four tissues. Strain-specific responses were identified in respect to C57BL/6 mice. Further species-specific responses were found (Swindell, et al., 2018).

The transcriptional response to DR have been studied for mRNAs widely, but not so much for microRNAs (miRNAs). miRNA response induced by DR can regulate important factors in processes such as longevity and ageing and is an integral component of the cellular response to DR (Orom, et al., 2012). DR induces a profound change in abundance of circulating miRNAs linked to growth and insulin signalling, indicating that miRNAs are involved in the mechanisms of DR. About 70% of the plasma miRNAs detected were conserved between monkey and humans (Schneider, et al., 2017). The microRNA expression in mouse breast tissue before and after DR have been characterized where several changes in the miRNA expression profiles were found. Specifically mir203 was found to be highly induced by DR. caveolin-1 as well as p63 are direct targets of mir203 *in vivo* during DR (Orom, et al., 2012).

DR is accompanied by a slowing of the progression of normal, age-related changes in transcript levels. DR resulted in the downregulation of genes primarly involved in cell growth, metabolism and reproduction (Pletcher, et al., 2002).

DNA methylation could play an important role in mediating the effects of DR because its sensitivity to the effects of nutrition and it can affect gene expression memory over time. Genome-wide changes in DNA

methylation, gene expression and lipidomics in response to DR and ageing in female mouse liver. DR is generally strongly protective against age-related DNA methylation changes. During ageing under DR, DNA methylation becomes targeted to gene bodies and is associated with reduced gene expression, specifically of genes involved in lipid metabolism (Hahn, et al., 2017). Methylomes from mice subjected to lifespan-extending conditions, including Prop1df/df dwarfism, DR or rapamycin were analysed in order to examine whether epigentic aging signatures are slowed down by these interventions. Mice treated with these lifespan-extending interventions were significantly younger in epigenetic age than untreated, wild type age-matched controls (Wang, et al., 2017).

Human maximum lifespan is substantially greater if compared to that of closely related primate species. The human transcriptome state, relative to other primate transcriptomes, does not match that of the DR mice or mice treated with resveratrol, but resembles the transcriptome state of *ad libitum* feed mice. Transcriptome changes induced by DR in mice are enriched among gene exhibiting age-related changes in primates, concentrated in specific expression patterns, and can be linked to defined functional pathways such as insulin signalling, cancer and immune response. Therefore the evolution of human longevity was likely independent of DR-induced lifespan extension mechanisms (Zhao, et al., 2014).

Expression of hundreds of genes exhibit a different response between young and old men upon DR. There is a downregulation of genes involved in the immune system in young but not old men. Immune response-related genes were higher expressed in old compared to young men. Most potential upstream regulators were controlling the immune response (Van Bussel, et al., 2016).

Physiological responses to DR are cell-type specific and variable throughout the lifespan. The influence of long-term DR on the CA1 hippocampal region was examined via mRNA sequencing and NanoString nCounter analysis. One year of DR feeding suppresses age-dependent changes of almost one thousand genes functionally associated with synaptic transmission including calcium signalling, long-term potential and Creb signalling. By comparing the influence of DR on transcriptional profiles at younger-adult and older-adult a conserved upregulation of proteome quality control and calcium buffering genes was identified. Expression of putative neuroprotective factors is also evaluated by DR in adulthood even though the global DR-specific expression profiles at younger and older age are highly diverged (Schafer, et al., 2015).

All those studies traditional utilized univariate methods for determining differential expression. Here in this work in contrary to previous work a multivariate approach is utilized to calculate differential expression for a greater amount of profiles. Using this approach tissue-common species-specific consensus signatures are generated for humans as well as the major biomedical model organisms. These consensus signatures are analysed for their significant associations. Moreover associations common to the signatures of all species considered here are derived as well.

Therefore, molecular consensus signatures [Molecular Profiling to Decipher Ageing] of dietary restriction in humans as well as model organisms were derived and sought for the major contributors and functional categories governing the effect of DR on ageing. More precisely, the effect of dietary restriction on the gene expression was extensively investigated in humans and various model organisms including rat, mouse, fly, nematode and yeast.

5.2 Methods

Gene expression signatures [Molecular Profiling to Decipher Ageing] were generated and characterized as described in [Ageing Signatures]. Briefly, gene expression profiles were downloaded from the NCBI Entrez Gene Expression Omnibus Gene File Transfer Protocol (FTP) server, parsed and annotated wit the respective platforms. Columns and rows that only had null/NaN (Not a Number) values were discarded. Other missing values were mean interpolated. Data was log2-transformed and quantile normalized if data was not yet log transformed and normalized. Here however, samples from dietary restriction individuals were contrasted via the Characteristic Direction with those on *ad libitum* diet to derive signatures representing the effect of dietary restriction on the transcriptome level [Figure 38 Dietary Restriction Signature]. The utilized datasets can be requested from the author. Differential expression was assessed with the characteristic direction method (Clark, et al., 2014) as detailed in [Ageing Signatures].

–

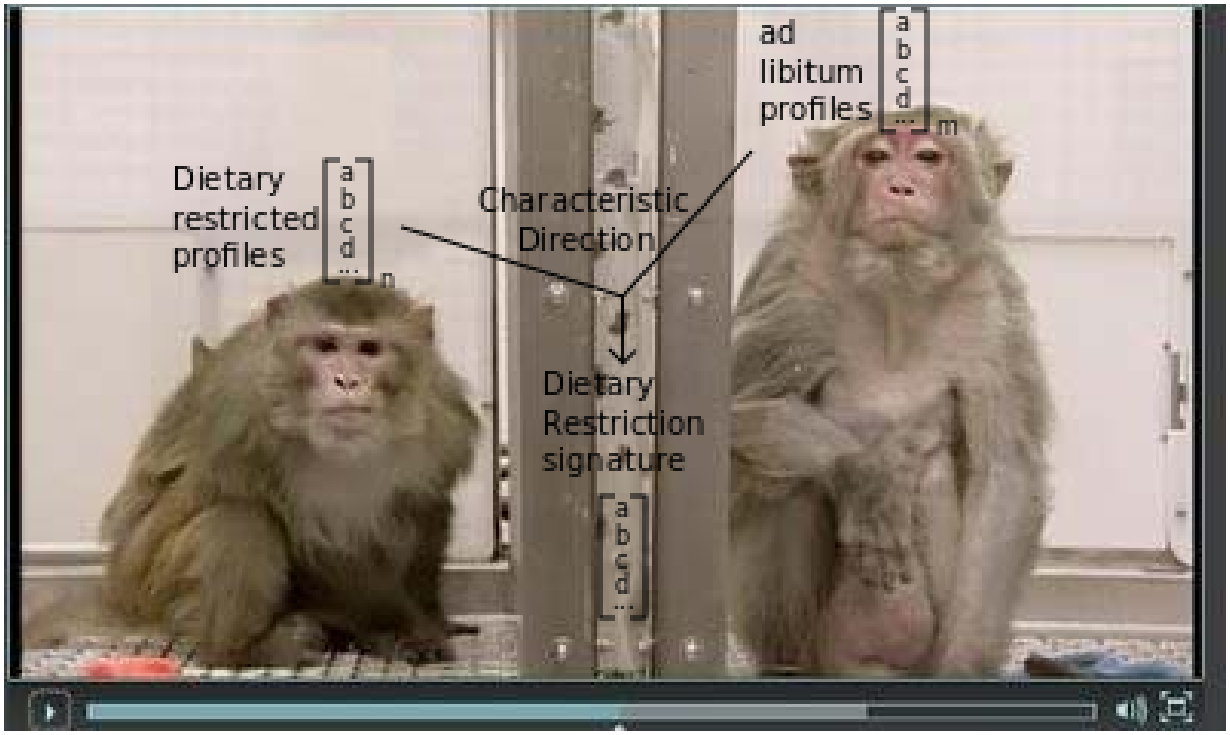

Figure 38: Dietary Restriction Signature.

Physical and genetic interactions were derived from BioGRID (Version 3.4.140) (Breitkreutz, et al., 2008), while processes, functions and locations were utilized from the Gene Ontology (Ashburner, et al., 2000) and downloaded from the NCBI FTP server. The Principal Angle Enrichment Analysis (Clark, et al., 2015) was applied for identifying significant associations. The p-values were corrected for multiple hypothesis testing via the Benjamini-Hochberg method (resulting in q-values) (Benjamini & HochBerg, 1995). The most significant associations of the up- and down-regulated and differentially expressed genes given a defined q-value cut-off were connected by the shortest path algorithm and graphed via automatic force-directed graph layout (Kobourov, 2012) as well as colour coded as described below. q-values were converted to node size scale by adding to the minimum size of 5 the negative of the exponent of the q-value divided by two.

Nodes were colour coded in the following way. Upregulated genes are filled red, downregulated genes are blue. Processes are green, functions are orange, and locations are yellow. Halos indicate respective significant associations with the same colour code. Nodes to be associated with upregulated and differential expressed genes get magenta coloured halos, those that are associated with downregulated and differential expressed genes obtain violet coloured halos and those that were found to be significant associated with upregulated as well as downregulated have halos coloured in purple. Physical interactions are pink, genetic interactions are green and other edges like, *involved in*, *exhibits* and *is located in* are grey. Node size represents significance. The methods used here are more detailed explained in [Ageing Signatures].

5.3 Results

5.3.1 Human

The significant associations (q-value < 5e-2) of genes that differentially expressed under dietary restriction in *Homo sapiens* are graphed as a network [Figure 39 H. sapiens Dietary Restriction].

Human DR-upregulated genes are significantly associated with participating in chromatin silencing and immune response, exhibit PolyA RNA binding and protein binding involved in protein folding, are located in nucleosome and nuclear chromatin, as well as interact with SPRED1. Human DR-downregulated genes significantly associate with participating in transcription from RNA polymerase II promoter, positive regulation of transcription DNA-templated, regulation of progesterone biosynthetic process, apoptotic process, NAD biosynthetic process, exhibit hemi-methylated DNA-binding and are located in Golgi apparatus. Human DR differentially expressed genes are significantly associated with participating in signal transduction, exhibiting DNA binding and being located in extracellular exosome.

–

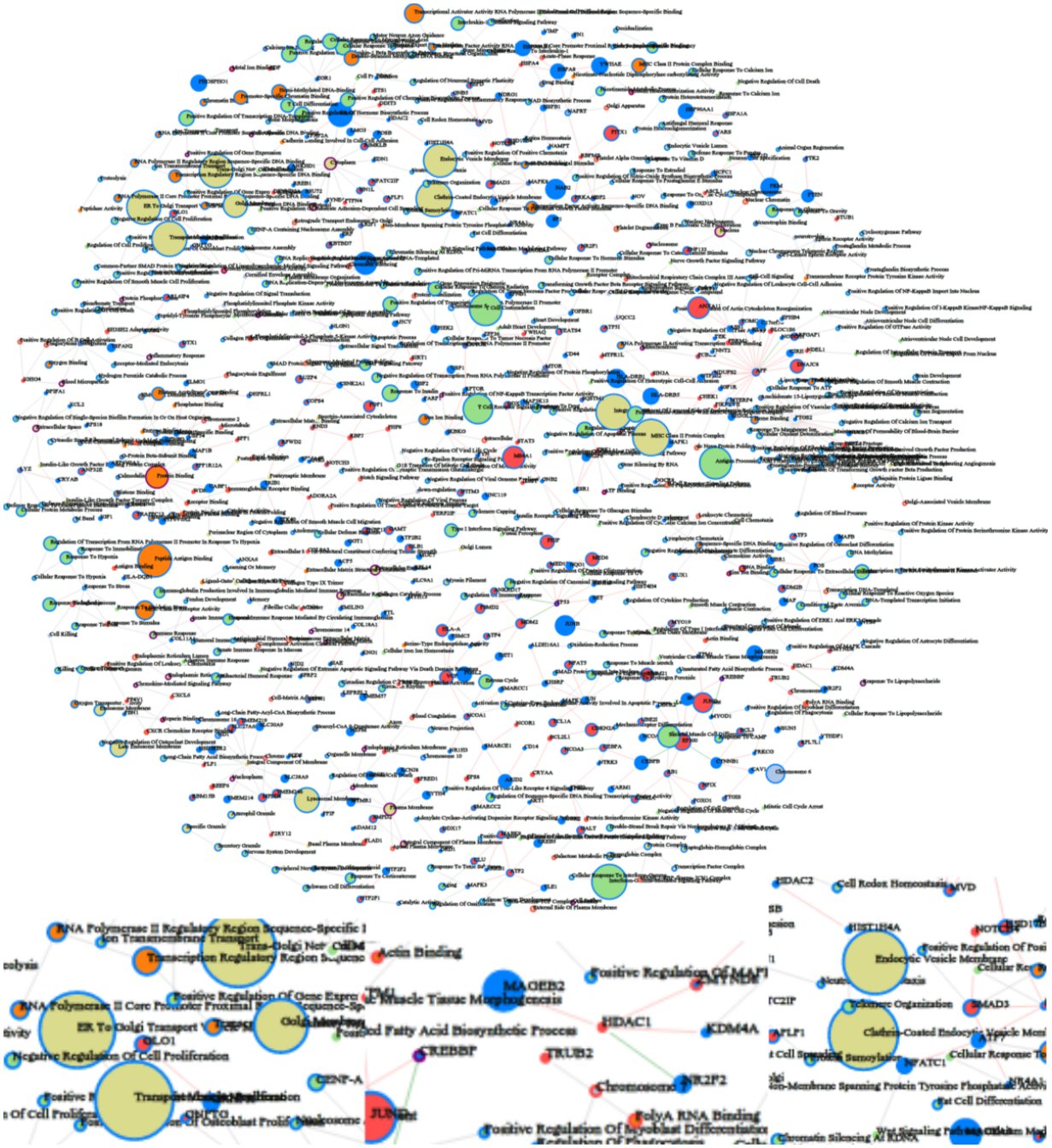

Figure 39: H. sapiens Dietary Restriction*. Network of genes differentially expressed upon DR in humans.*

–

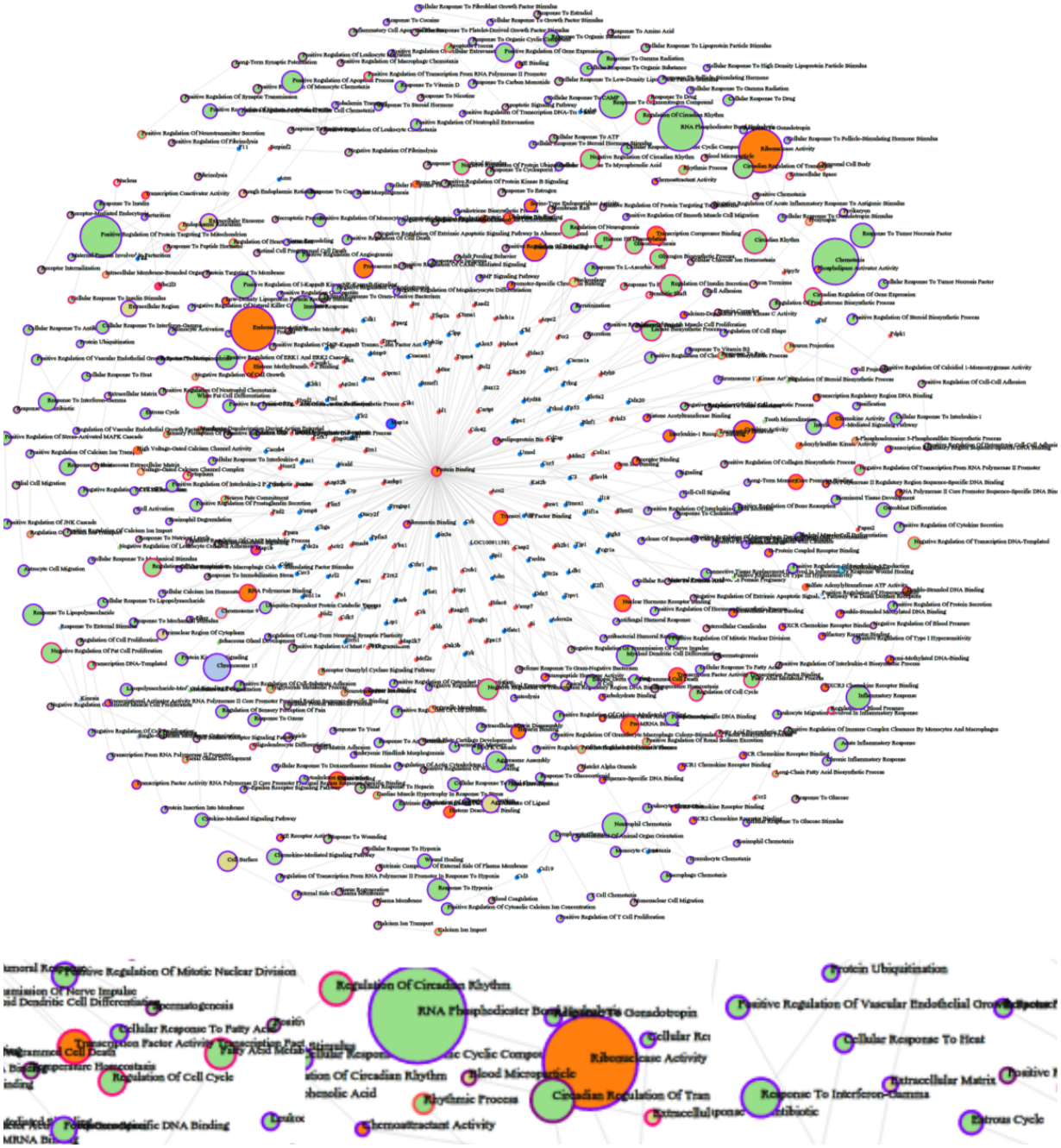

Figure 40: R. norvegicus Dietary Restriction*. Network of genes differentially expressed upon DR in rat.*

5.3.2 Rat

Rattus norvegicus is the classic model organisms in which dietary restriction was discovered originally (McCay, 1933). Gene expression profiles of rats under *ad libitum* fed and under dietary restriction conditions were used to derive a consensus signature of dietary restriction for the rat. The significant associations (q-value < 5e-10) with this signatures are graphed [Figure 40 R. norvegicus Dietary Restriction].

Rat DR-upregulated genes are significantly associated with participating in circadian rhythm, negative regulation of circadian rhythm, negative regulation of transcription regulatory region DNA binding, histone H3 deacetylation, and regulation of neurogenesis, exhibiting histone methyltransferase binding, transcription factor binding and ubiquitin binding, and being located in the perinuclear region of cytoplasm. Rat DR-downregulated genes are significantly associated with participating in ageing, positive regulation of protein targeting to the mitochondrion, positive regulation of I-kappaB kinase NF-KappaB signalling, inflammatory response, positive regulation of apoptotic process, aggresome assembly, exhibiting chemokine activity, and being located in extracellular exosome, as well as the aggresome.

–

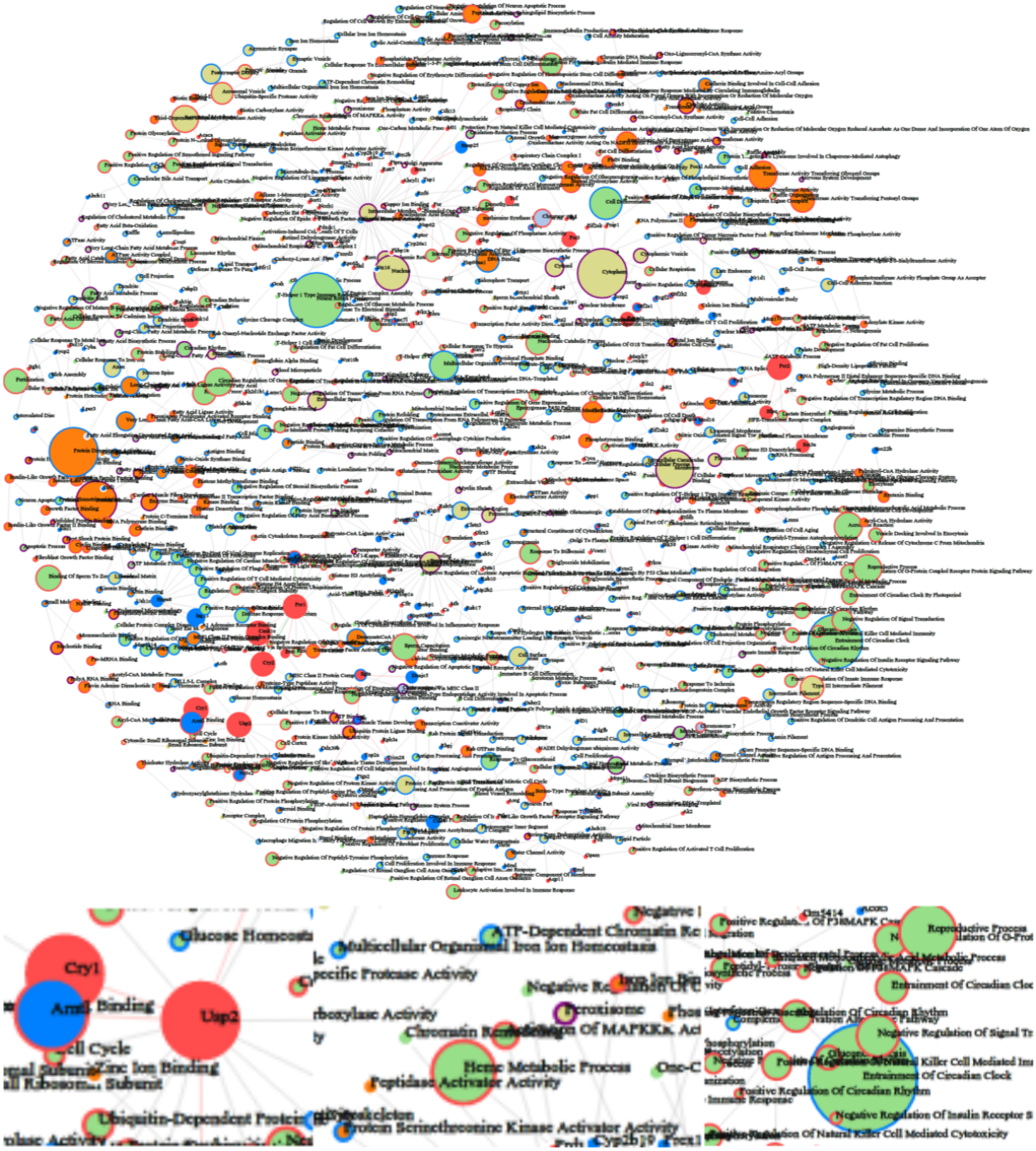

Figure 41: M. musculus Dietary Restriction*. Network of genes differentially expressed upon DR in mouse.*

5.3.3 Mouse

Mus musculus is another rodent model organisms that has been extensively used for studying the lifespan extending effect of DR and a plethora of gene expression profiles are publicly available. Murine gene expression profiles have been combined into a molecular signature of ageing in mouse and assessed for significant associations (q-value < 5e-5) [Figure 41 M. musculus Dietary Restriction].

Mouse DR-upregulated genes are significantly associated with participating in circadian regulation of gene expression, reproductive process, rhythmic process, negative regulation of cysteine-type endopeptidase activity involved in apoptotic process, and histone H3 deacetylation. Mouse DR-downregulated genes are significantly associated with participating in entrainment of circadian clock, Notch signalling pathway, cell differentiation, multicellular organism development, negative regulation of transcription from RNA polymerase II promoter, and chaperone mediated protein folding requiring cofactor. Mouse DR-differentially expressed genes are significantly associated with participating in circadian rhythm, transcription DNA-templated, being located in extracellular exosome, perinuclear region of cytoplasm, mitochondrion, and mitochondrial inner membrane.

–

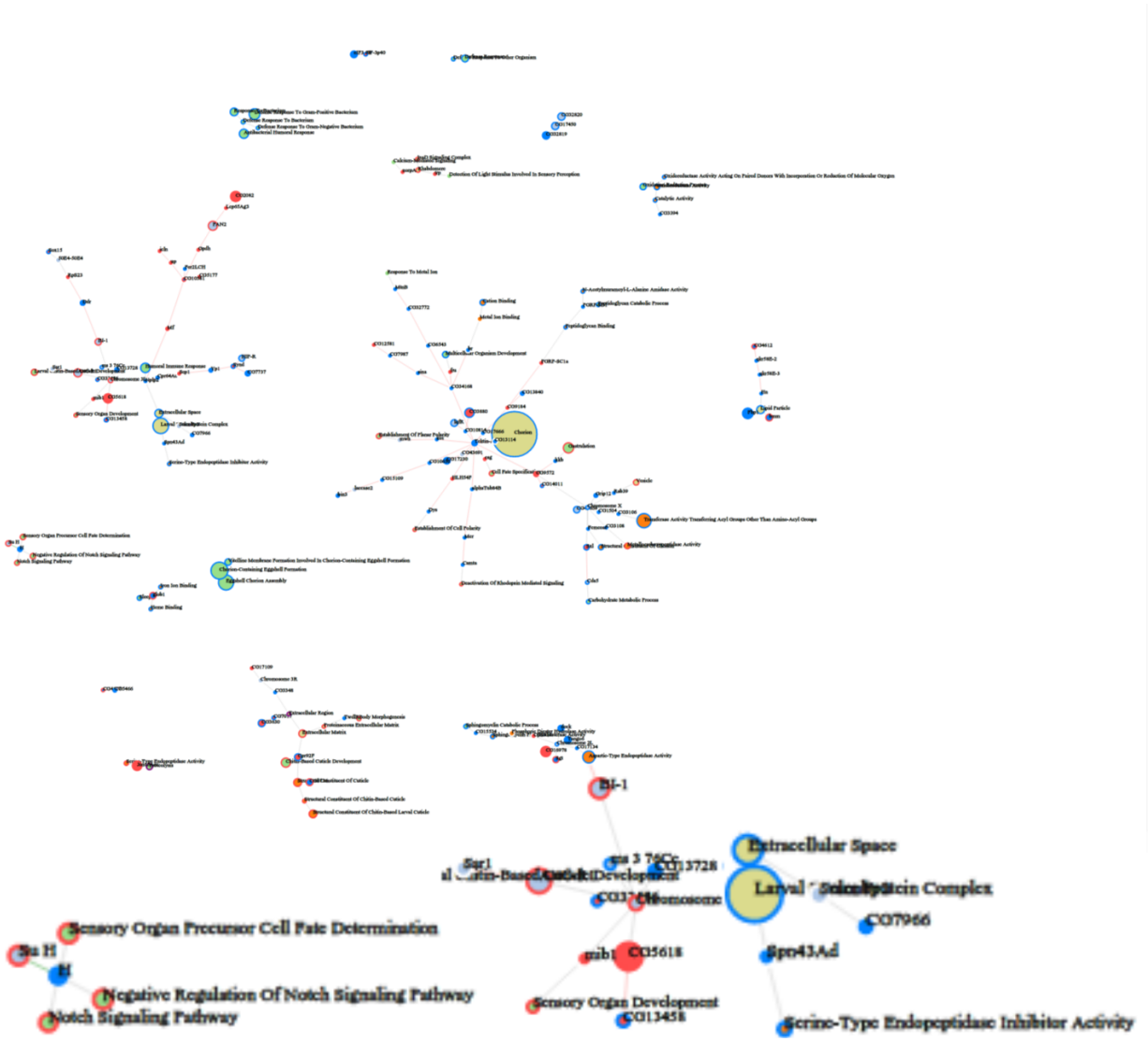

Figure 42: D. melanogaster Dietary Restriction*. Network of genes differentially expressed upon DR in fruit fly.*

5.3.4 Fly

Drosophila melanogaster have been profiled for the effect of dietary restriction protocols on the gene expression level. The consensus signature of dietary restriction in fruit fly has been investigated for significant associations (q-value < 5e-2) and graphed here [Figure 42 D. melanogaster Dietary Restriction].

–

Figure 43: C. elegans Dietary Restriction*. Network of genes differentially expressed upon DR in nematode.*

Fly DR-upregulated genes are significantly associated with participating in negative regulation of Notch signalling pathway and body morphogenesis. Fly DR-downregulated genes are significantly associated

with participating in humoral immune response in chorion and exhibiting transferase activity transferring acyl groups other than amino-acyl groups. Fly DR-differentially expressed genes are significantly associated with participating in proteolysis.

–

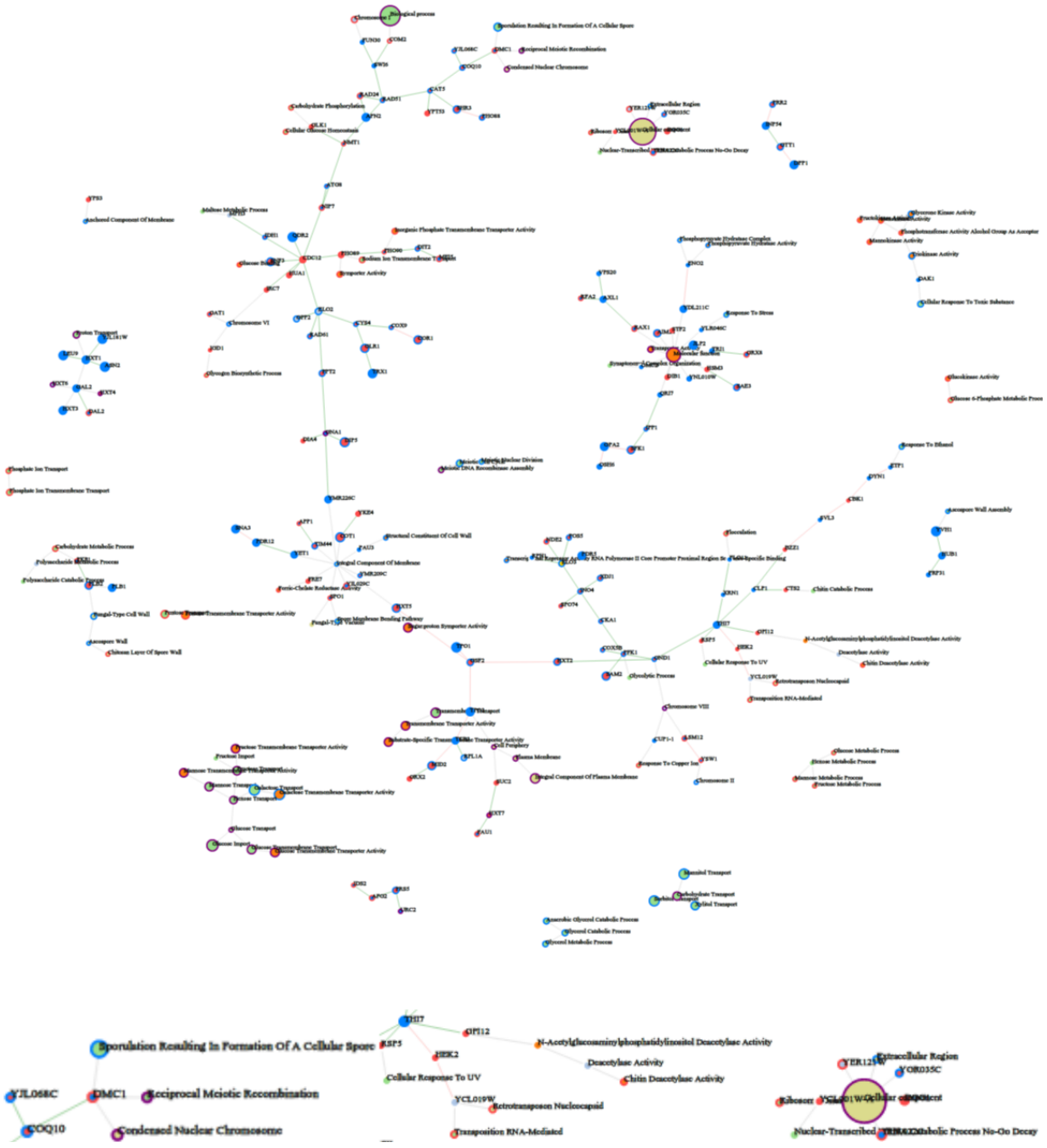

Figure 44: S. cerevisiae Dietary Restriction*. Network of genes differentially expressed upon DR in budding yeast.*

5.3.5 Worm

Caenorhabditis elegans have been gene expression profiled under dietary restriction regimens. From those a consensus signature of representing dietary restriction in nematode have been established. The significant associations (q-value < 5e-2) found in this signature have been graphed [Figure 43 C. elegans Dietary Restriction].

Worm DR-upregulated genes are significantly associated with participating in determination of adult lifespan, proteolysis, and fatty acid metabolic process and exhibiting NAD binding and being located in nucleosome. Worm DR-downregulated genes are significantly associated with participating in innate immune response, cystathionine gamma-synthase activity, proteolysis involved in cellular protein catabolic process and exhibiting hormone activity. Worm DR-differentially expressed genes are significantly associated with being located in lysosome.

5.3.6 Yeast

Saccharomyces cerevisiae under different levels of glucose have been profiled. A consensus signature of dietary restriction in budding yeast have been created and subjected to Principle Angle Enrichment Analysis with a q-value threshold of 5e-5 [Figure 44 S. cerevisiae Dietary Restriction].

Yeast DR-upregulated genes are significantly associated with participating in meiotic DNA recombinase assembly, transposition RNA-mediated and being located in retrotransposon nucleocapsid. Yeast DR-downregulated genes are significantly associated with participating in response to stress, sporulation resulting in formation of a cellular spore, meiotic cell cycle, ribosome disassembly, exhibiting transcriptional repressor activity RNA polymerase II core promoter proximal region sequence-specific binding and being located in condensed nuclear chromosome.

5.3.7 Common DR-Signature Across Species

The consensus signature of human, rat, mouse, fly, worm and yeast have been subjected to Principle Angle Enrichment Analysis individually and results combined. The common associations are graphed [Figure 45 Common Dietary Restriction].

DR-upregulated genes are significantly associated with participating in transcription DNA-templated and DNA-templated negative regulation of transcription as well as being located in nucleus and nucleoplasm [Figure 45 Common Dietary Restriction]. DR-downregulated genes are significantly associated with participating in response to glucocorticoid, positive regulation of cell death, positive regulation of ERK1 and ERK2 cascade, and positive regulation of gene expression, exhibiting transcription factor binding and being located in extracellular exosome [Figure 45 Common Dietary Restriction]. DR-differentially expressed genes are significantly associated with participating in response to stress, circadian rhythm, apoptotic process, lysosome and proteolysis [Figure 45 Common Dietary Restriction].

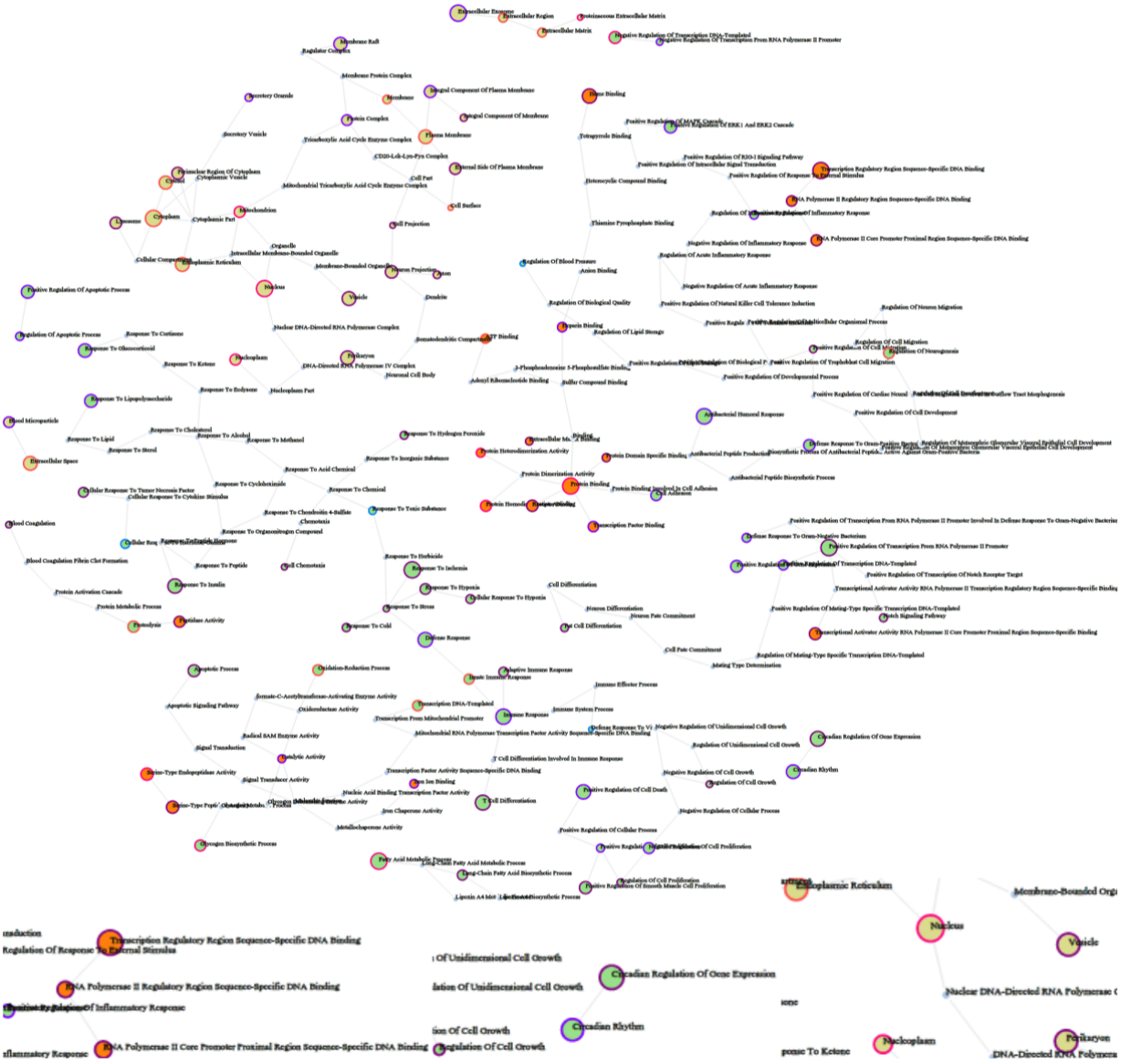

Figure 45: Common Dietary Restriction. *Network of associations with DR-differentially expressed genes commonly shared between multiple species.*

5.4 Discussion

Molecular signatures representing dietary restriction, an intervention known to modify the basic ageing process, were derived for humans as well as common biomedical model organisms. Consensus signatures were created and characterised for their significant associations from various sources.

5.4.1 Ageing and Lifespan Determination

DR-differentially expressed genes are significantly associated with being involved in cell ageing and in particular positive regulation of cell ageing [Mouse]. DR-upregulated genes are significantly associated with participating in determination of adult lifespan [Figure 43 C. elegans Dietary Restriction] and being involved in chronological cell ageing [Rat], while DR-downregulated genes are commonly significantly associated with participating in ageing [Human; Figure 40: R. norvegicus Dietary Restriction; Common DR-Signature Across Species] which may mean that DR upregulates ageing-suppressor genes and downregulates gerontogenes in order to mediate its anti-ageing effect.

Further DR-downregulated genes are significantly associated with being involved in cellular senescence [Mouse]. DR reduces senescence cell frequency (Wang, et al., 2010).

5.4.2 Chromatin

DR-upregulated genes are significantly associated with participating in chromatin silencing and being located in nucleosome and nuclear chromatin [Figure 38 H. sapiens Dietary Restriction]. DR-upregulated genes are significantly associated with chromatin silencing, while DR-downregulated genes are significantly associated with chromatin silencing at rDNA [Human].

During ageing, changes in chromatin state that alter the gene transcription have been postulated to cause the expression of genes that are normally silenced, leading to deleterious age-related effects on cellular physiology. Loss of silencing with age has been well documented (Sedivy, et al., 2008; Berger, 2007; Dang, et al., 2009; Feser & Tyler, 2011; Han & Brunet, 2012; De Cecco, et al., 2013; Kim, et al., 1999; Smeal, et al., 1996; Wood, et al., 2010; Maxwell, et al., 2011). Dietary restriction delays the age-related loss of gene silencing, suggesting that the loss of gene silencing is a component of normal ageing (Jiang, et al., 2013). DR-downregulated genes are commonly significantly associated with exhibiting hemi-methylated DNA-binding as well as double-stranded methylated DNA-binding [Common DR-Signature Across Species]. DR counteracts the decrease of DNA methylation at promoter and intron regions (Kim, et al., 2016). Further supporting this, it was found that genes that respond to dietary restriction also exhibit age-related changes in DNA methylation (Ions, et al., 2013).

DR-upregulated genes are significantly associated with participating in histone H3 deacetylation, negative regulation of transcription regulatory region DNA binding, histone H3 deacetylation and exhibiting methyltransferase binding [Figure 40 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction]. H3K2me3, H3R2me2, H3K79me3, and H4K20me2 tend to disappear with age but are partially restored by both DR and rapamycin treatment. However both DR and rapamycin treatment also have significant impact on H3K27ac, H4K16ac, H4R2me2 and H3K56ac which do not change as the animal ages (Gong, et al., 2015).

DR-upregulated genes are significantly associated with participating in RNA-mediated transposition and being located in retrotransposon nucleocapsid [Figure 44 S. cerevisiae Dietary Restriction]. Transpositional elements are mobile genetic elements, that are highly enriched in heterochromatin and constitute a large percentage of the DNA content of eukaryotic genes. Transcripts of many genes native to heterochromatic regions and transpositional elements increase with age, while dietary restriction represses the age-related increase (Wood, et al., 2016).

5.4.3 RNA Interference

DR-differentially expressed genes are significantly associated with pre-miRNA processing [Worm]. DR-downregulated genes are significantly associated with gene silencing by RNA as well as positive regulation of pri-miRNA transcription from RNA polymerase II promoter [Human].

This agrees with the findings that the knockout of specific microRNAs extend the lifespan via dietary restriction (Vora, et al., 2013). Even more important is the observation that microRNA are actually necessary for the lifespan extending effect of DR (Smith-Vikos, et al., 2014).

5.4.4 Protein Homeostasis

DR differentially expressed genes are commonly significantly associated with participating in proteolysis and being located in proteasome and lysosome [Figure 42 D. melanogaster Dietary Restriction; Figure 41 C. elegans Dietary Restriction; Figure 45 Common Dietary Restriction].

DR-upregulated genes are significantly associated with participating in protein folding [Figure 39 H. sapiens Dietary Restriction] while DR-downregulated genes are significantly associated with exhibiting chaperone mediated protein folding requiring a cofactor [Figure 41 M. musculus Dietary Restriction]. The heat shock proteins are responsible for correct protein folding. They are classified HSPs with chaperone functions (five classes) and small heat shock proteins, which are mostly cofactors of the larger HSPs. The primary chaperone HSPs are HSP60, HSP70 and HSP90. There are mitochondrial versions of these called mortalin (mitoHSP70) and TRAP-1 (HSP90). Several chaperones are encoded by ageing-suppressor genes. The folding and assembly of proteins is essential for protein function, the long-term health of the cell, and longevity of the organism (Karady, et al., 2013). DR-downregulated genes are significantly associated with participating in aggresome assembly and being located in the aggresome [Figure 40 R. norvegicus Dietary Restriction]. Dietary restriction seems to reduce the load of protein misfolding and damage. Indeed dietary restriction was found to confer a general protective effect against proteotoxicity and promotes longevity by a mechanisms involving the heat shock factor (Steinkraus, et al., 2008b). HSF1 was found to have a positive characteristic direction in the consensus signature of dietary restriction of human, mouse and rat [Human; Rat; Mouse].

DR-upregulated genes are significantly associated with participating in ubiquitin binding [Figure 40 R. norvegicus Dietary Restriction]. Ageing increases the levels of ubiquitinated proteins and dietary restriction significantly reduces these age-related increases (Zhang, et al., 2007).

5.4.5 Hormone

DR-downregulated genes are significantly associated with exhibiting hormone activity [Figure 39 H. sapiens Dietary Restriction; Figure 40 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction; Figure 43: C. elegans Dietary Restriction]. Dietary restriction is well known to exhibit a systemic effect via modulating hormonal signalling including certain endocrine systems (Herlihy, et al., 1990). Several hormones and associated signalling mediators encoded by gerontogenes such as those related to growth hormone, insulin-like growth factor and insulin among others are commonly reduced upon dietary restriction (Chiba, et al., 2007).

DR-upregulated genes are significantly associated with participating in response to glucocorticoid [Figure 45 Common Dietary Restriction]. Elevated plasma glucocorticoid levels have a causal role in rapid deterioration following reproduction in semelparous vertebrate species. This is because they induce a Cushing type syndrome to mobilise protein in order to support the once cycle of reproduction. Glucocorticoids can promote ageing in vertebrate species. However periods of moderately elevated plasma glucocortcoid levels may retard the ageing process in mammals (Masoro, 1995).

DR-downregulated genes are significantly associated with participating in regulation of progesterone biosynthetic processes [Figure 39 H. sapiens Dietary Restriction]. Progesterone administration enhances memory in aged mice (Lewis, et al., 2008) independent of the progesterone receptor (Frye & Walf, 2010).

5.4.6 Signalling

Notch signalling pathway and more specifically negative regulation of Notch signalling pathway is associated with DR-downregulated genes [Figure 41 M. musculus Dietary Restriction; Figure 42 D. melanogaster Dietary Restriction]. The Notch signalling pathway was also found to be downregulated upon resveratrol supplementation that is a DR mimetic (Konings, et al., 2014).

Surprisingly common to DR-upregulation is a very specific association to positive regulation of the ERK1 and ERK2 cascade. This could mean that DR enhances MAPK signalling [Figure 45 Common Dietary Restriction].

5.4.7 Metabolism

Response to fatty acid and unsaturated fatty acid biosynthetic process are commonly significantly associated with DR-differentially expressed genes. Fatty acid metabolic process, fatty acid biosynthetic process, and long-chain fatty acid biosynthetic process are commonly upregulated [Figure 39 H. sapiens Dietary Restriction; Figure 40 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction; Figure 42 C. elegans Dietary Restriction; Figure 45 Common Dietary Restriction].

Fatty acid chain length and susceptibility to oxidation correlates with longevity (Shmookler Reis, et al., 2011). The amount and type of dietary fatty acids can also profoundly affect lifespan (Jolly, et al., 2001). The effect of a restricted diet to modify the proton leak and membrane potential as well as reactive oxygen species generation rate can be stimulated by a range of non-esterified free fatty acids acting on the adenine nucleotide translocase to enhance protonophoric activity. Adenine nucleotide translocase is the dominant proton leak channel induced under dietary restriction. Therefore mobilised free fatty acids in mitochondria of dietary restriction fed animals enhance the proton leak and reduce reactive oxygen species generation (Ash & Merry, 2011).

DR downregulated genes are significantly associated with cystathionine gamma-synthase activity [Figure 43 C. elegans Dietary Restriction] as well as *de novo* L-methionine biosynthetic process [Worm]. DR-upregulated genes are significantly associated with methionine synthase reductase activity [Mouse].

The transsulfuration pathway is a highly conserved mechanism of metabolising sulfur-containing amino acids, methionine and cysteine. Cystathionine beta-synthase is encoded by an ageing-suppressor gene and DR-essential (Kabil, et al., 2011). Restriction of methionine is sufficient to extend lifespan (Johnson & Johnson, 2014).

DR-upregulated genes exhibit NAD binding [Figure 43 C. elegans Dietary Restriction] and DR-downregulated genes are significantly associated with participating in NAD biosynthetic metabolism [Figure 39 H. sapiens Dietary Restriction]. DR involves a NAD(+)-dependent mechanism as well as a shift toward oxidative metabolism (Moroz, et al., 2014).

5.4.8 Reproduction

DR-upregulated genes are significantly associated with participating in reproductive process [Figure 41 M. musculus Dietary Restriction; Figure 41 S. cerevisiae Dietary Restriction]. Overexpression of reproduction-related transcription factors is capable of reversing ageing (Unal, et al., 2011).

5.4.9 Apoptosis

DR-upregulated [Figure 41 M. musculus Dietary Restriction; Figure 45 Common Dietary Restriction] and DR-downregulated genes are significantly associated with participating in apoptotic process [Figure 39 H. sapiens Dietary Restriction; Figure 45 Common Dietary Restriction]. DR is known to favour apoptosis over proliferation (Dunn, et al., 1997).

Dietary restriction induces the apoptosis of malignant cells and may also induces apoptosis of senescence cells as dietary restriction reduces senescence cell number. Cancer incidence increases progressively with age. Interventions that retard the rate of ageing simultaneously retard the incidence of many forms of cancer. Dietary restriction especially is known to be a non-invasive manipulation that retards most physiological and molecular indices of ageing as well as the incidence of spontaneous and chemically induced tumours (Muskhelishvili, et al., 1995; Wachsman, 1996). Adult-onset, short-term DR reduces frequencies of senescence cells (Wang, et al., 2010). While DR removes malignant and senescence cells via apoptosis, it does prevent cell death of healthy cells such as neurons (Shruthi, et al., 2016).

5.4.10 Golgi

DR-upregulated genes are commonly significantly associated with being located in the Golgi Apparatus [Common DR-Signature Across Species]. The volume of the Golgi apparatus perikaryon increases significantly with age. The mean percentage of perikaryal volume occupied by the Golgi apparatus decreases with age (Ledda, et al., 2001).

Specifically DR-upregulated genes are significantly associated with retrograde transport endosome to the Golgi apparatus [Human] that is the directed movement of membrane-bounded vesicles from endosomes back to the trans-Golgi network. It is possible that misfolded proteins are send back to the Golgi Apparatus for refolding.

5.4.11 Stem Cells

DR-upregulated genes are commonly significantly associated with being involved in regulation of neurogenesis [Figure 40 R. norvegicus Dietary Restriction; Common DR-Signature Across Species], where negative regulation of neurogenesis specifically is significantly associated with DR-downregulated genes [Rat]. Dietary restriction promotes adult neurogenesis (Kitamura, et al., 2006).

DR downregulated genes are significantly associated with stem cell division [Rat]. Positive regulation of hematopoietic stem cell proliferation is significantly associated with differentially expressed genes during ageing [Rat] and negative regulation of hematopoietic stem cell differentiation is associated with ageing-upregulated genes [Mouse]. Hematopoietic senescence is postponed and stem cell function is enhanced by DR (Chen, et al., 2003).

5.4.12 Inflammation

DR-upregulated genes are significantly associated with participating in the immune response [Figure 39 H. sapiens Dietary Restriction]. Food restriction delays the loss of several cellular immune functions (Byun, et al., 1995).

DR-downregulated genes are significantly associated with participating in positive regulation of I-kappaB kinase NF-KappaB signalling and inflammatory response, as well as exhibiting chemokine activity [Figure 40 R. norvegicus Dietary Restriction]. Dietary restriction downregulates NF-kappaB (Singh, et al., 2015). DR downregulates genes associated with innate immunity [Figure 43 C. elegans Dietary Restriction]. A reduction in chronic inflammation is widely observed under dietary restriction feeding conditions (Ye & Keller, 2010).

5.4.13 Mitochondria

DR-differentially expressed genes are significantly associated with mitochondria [Figure 39 H. sapiens Dietary Restriction; Figure 39 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction; Figure 43 C. elegans Dietary Restriction]. DR-downregulated genes are significantly associated with participating in positive regulation of protein targeting to the mitochondrion [Figure 40 R. norvegicus Dietary Restriction].

The mitochondrion is a major hub in the gene expression programme under lifespan-promoting interventions such as dietary restriction, genetic DR mimetics, heat shock and hydrogen peroxide (Sharma, et al., 2011). Mitochondria enlarge under dietary restriction feeding and dietary restriction retards the age-dependent loss of mitochondria (Weindruch, et al., 1980).

Response to reactive oxygen species is commonly associated with genes upregulated during DR [Common DR-Signature Across Species]. Also positive regulation of superoxide anion generation and positive regulation of membrane potential are significantly associated with genes differentially expressed upon DR [Rat].

Restricted feeding regimens that extend the lifespan lower the rate of mitochondrial reactive oxygen species generation. Mitochondria from different tissues adapt to DR feeding with a lowered membrane potential that results from an enhanced proton leak (Ash & Merry, 2011).

5.4.14 Circadian Rhythm

DR-differentially expressed genes, DR-downregulated genes and DR-upregulated genes are significantly associated with controlling the circadian rhythm, circadian regulation of gene expression and rhythmic process [Figure 39 H. sapiens Dietary Restriction; Figure 40 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction; Figure 45: Common Dietary Restriction]. This may be interpreted as that the circadian clock is modulated by DR. Circadian rhythms are generated by an intrinsic cellular mechanisms (involving transcriptional feedback loops) that controls a large array of physiological and metabolic processes.

There is an erosion in the robustness of circadian rhythms during ageing. Further disruption of the clock by genetic ablation of specific genes is associated with ageing-related phenotypes. Intracellular pathways including nutrient sensors impinge cellular and epigenetic mechanisms that control the ageing processes also control the circadian clock (Orozco-Solis+Sassone-Corsi, 2014).

5.4.15 Growth/Differentiation/Development

DR downregulates the regulation of cell growth and differentiation. Further, DR downregulates genes are commonly associated with multicellular organism development [Figure 41 M. musculus Dietary Restriction; Common DR-Signature Across Species].

Dietary restriction retards growth and development (Masoro, 1996). If ageing is an extension of development, DR may retard ageing by retarding the developmental program that drives ageing.

5.4.16 Stress Response

DR commonly and significantly upregulates genes involved in cellular responses to (oxidative) stress, the response to osmotic stress, and fluid shear stress and atherosclerosis [Figure 45 Common Dietary Restriction; Common DR-Signature Across Species]. Further DR-downregulated genes are significantly associated with oxidative stress induced senescence, and DNA damage/telomere stress induced senescence [Human].

It is well known that chronic stress and prolonged activation of defence pathways have deleterious consequences for the cell (Lin, et al., 2007; Walter & Ron, 2011; Lamech & Haynes, 2015; Roth, et al., 2014). Dietary restriction is shown to be beneficial as it induces the cellular stress response machinery (Bhadra, et al., 2016).

5.4.17 Gene Regulation

DR upregulated as well as DR downregulated genes are commonly associated with processes, activities and location related to gene regulation, especially at the level of transcription [Figure 39 H. sapiens Dietary Restriction; Figure 40 R. norvegicus Dietary Restriction; Figure 41: M. musculus Dietary Restriction; Figure 41 C. elegans Dietary Restriction; Figure 42: S. cerevisiae Dietary Restriction; Figure 45 Common Dietary Restriction]. This indicates that transcription factors could be identified that exerts the ageing retarding effect of dietary restriction. DR downregulated genes are commonly significantly associated with epigenetic regulation of gene expression [Common DR-Signature Across Species]. DR up- and down-regulated genes are both commonly significantly associated with circadian regulation of gene expression [Common DR-Signature Across Species].

Gene regulation is one mechanisms underlying the response to dietary restriction (Ions, et al., 2013). It may modulate the circadian clock, silence genes epigenetically and utilize specific transcription factors to exert ageing counteracting mechanisms.

Previous attempt to establish a consensus signature of dietary restriction found overexpression of lipid metabolism and circadian rhythm, and underexpression of sterol biosynthesis and innate immune response (Plank, et al., 2012). Here metabolism of lipids and lipoproteins as well as lipid biosynthetic process are found to be commonly associated with genes upregulated upon DR [Common DR-Signature Across Species]. Circadian clock was identified here as modulated by DR differential expression

[Circadian Rhythm]. Genes associated with sterol biosynthesis are downregulated in DR conditions [Mouse]. Further, while the innate immune response seems to be modulated (commonly significantly associated with both up- and down-regulated genes upon DR) positive regulation of inflammatory response was found to be commonly associated with DR-downregulated genes [Common DR-Signature Across Species].

In future extension of the work presented here different regimens could be differentiated (e.g. different strengths of DR and types of DR). Also the overlap of those signatures with the respective ageing signatures could be explored.

As dietary restriction has been assumed to work via a common mechanism across taxa, there might be a common consensus signature representing the influence of dietary restriction on the gene expression patterns shared among phylogenetically distinct species.

5.5 Conclusion

Dietary restriction affects the transcriptome commonly by modulating ageing genes directly, as well as those associated with chromatin, protein homeostasis, specific hormones, Notch signalling, metabolism of fatty acids, methionine and NAD, reproduction-related genes, apoptosis, inflammation, neurogenesis, mitochondria and Golgi, as well as the circadian rhythm, developmental genes, stress response, and commonly gene expression regulation.

6 Small Molecule Predictions

Abstract: Small molecules with the potential to intervene with the ageing process were identified via a variety of approaches. Guilt-by-association applied on the drug-protein/gene interactions and gene signature (list of genes with corresponding differential expression metrics) matching was utilized to identify potential drugs that can slow down or reverse ageing. This method was able to retrieve known as well as novel ageing interfering drug candidates.

6.1 Background

Thousands of genes in model organisms and some in humans are known to effect the lifespan [Ageing Genes]. More and more small molecules that extend longevity similar to genetic perturbations are been discovered. Many small molecules are recognised that exert their effect by affecting specific targets that are for instance proteins encoded by genes. Here it was hypothesized that by utilizing knowledge about distinct classes of ageing genes drugs can be identified that are potential geroprotectors. Additionally ageing and interventions that impact on ageing trigger distinct patterns of gene expression level (so-called molecular signatures) [Molecular Profiling to Decipher Ageing]. This can be leveraged to identify small molecules that mimic or reverse certain biological states, conditions, or processes [Anti-Ageing Drugs]. Here it is sought to find drugs that interfere with the ageing process by performing gene signature matching. Specifically here small molecules were identified that for example reverse age-related gene expression changes or mimic lifespan-extending interventions (such as dietary restriction) at the gene expression level.

Ageing counteracting drugs can be identified by utilizing signatures associated with ageing and drug signatures that either reverse or mimic those effects at the level of the transcriptome. Anti-ageing drugs can be found by gene expression matches that either reverse ageing, HGPS, cellular senescence, and age-related diseases or mimic the effect of dietary restriction as well as long-lived mutants. *In silico* methods for screening and ranking possible geroprotectors were developed. For this, changes in the activated and suppressed pathways involved in ageing and longevity can be constructed using the gene expression and epigenetic profiles of tissue from young and old individuals. Possible interventions are selected and rated according to their ability to regulate age-related changes and minimize the differences in signalling (Zhavoronkov, et al., 2014). Public gene expression repositories can be transformed into disease diagnosis databases. Such a database can be used to construct a disease-drug connectivity map. The statistical significance of the link between a disease and a drug is based on the intersection of the disease-related genes and drug-related genes using for instance a hypergeometric test (Huang, et al., 2010).

Compound signatures can be detected on the Connectivity Map (CMap) which provides gene expression profiles of cell cultures incubated with various compounds (Lamb, 2007). The Connectivity Map is a large-scale compendium of functional perturbations in cultured human cells that are coupled to a gene-expression readout. L1000 is a new gene expression profiling method that dramatically lowers cost of generating profiles by only probing for around 1000 "landmark" genes and inferencing the expression of others. The Connectivity Map includes over 1.3 million publicly accessible L1000 profiles. The CMap facilitates the discovery of connections between genes, drugs, and diseases as well as small-molecule mechanism and annotation of genetic variants (Subramanian, et al., 2017). Various substances such as drugs, dietary supplements, nanomaterials, metabolites and hormones that have shown to extend the lifespan have also been transcriptionally profiled. This work establishes feasibility and utility of a truly comprehensive small molecule predictions for targeting ageing.

6.2 Methods

Guilt-by-association, as described previously [Ageing Genes], was applied to drug-protein/gene interventions derived from the Drug Gene Interaction Database (Griffith, et al., 2013). Human homologs of ageing genes or specific subclasses of ageing genes were used as seeds. Homologous genes were retrieved from NCBI HomoloGene via the File Transfer Protocol. The hypergeometric test was used and corrected for multiple hypothesis testing via the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). A significance threshold of 0.05 as q-value was utilized for generating the seed drugs of the network.

Physical and genetic interactions of genes/proteins were utilized from BioGRID (Version 3.4.140) (Breitkreutz, et al., 2008). Briefly here networks were generated by retrieving the first degree interactors plus applying shortest path for connecting the unconnected nodes. Shortest path was calculated between each unconnected node and all other nodes where the input of the shortest path algorithm is as source each unconnected node and as target all other connected nodes and from this calculating the shortest path between the unconnected node and all other connected nodes. Nodes representing drugs are coloured pink, gerontogenes coloured red and ageing-suppressors are coloured blue while those that are were found to be both gerontogene and ageing-suppressor gene are brown. Other nodes without known association are grey. Molecular signatures [Molecular Profiling to Decipher Ageing] were generated as described in [Ageing Signatures]. Briefly, here compounds gene expression profiles (which are gene expression profiles of cells in culture that were supplemented with that specific compound derived from the Connectivity Map (Lamb, 2007)) were used to derive small molecule/drug signatures (by processing gene expression profiles and calculating differential expression metrics including characteristic direction (Clark, et al., 2014) for each gene, as described previously [Ageing Signatures]. Each drug treatment was applied to a cell type with a particular dose for a defined duration. Datasets can be requested from the author. Those signatures were used in combination with previous derived signatures about ageing [Ageing Signatures] and dietary restriction [Dietary Restriction Signatures] to find (via pattern matching based on cosine similarity) drugs that reverse ageing or mimic the effect of lifespan-extending interventions. The score was calculated from the cosine similarity according to:

```
similarity = cos(0) = A × B / (||A||||B|||)
```

Where A and B are the vectors to be compared with each other. Positive score indicates mimicking, while a negative score indicates reversing/opposing [Figure 46 Molecular Signature Matching]. Reversing is the reverse of similarity (i.e. distance). Results are visualized as graphs by applying force-directed graph layout (Kobourov, 2012) and coloured as described in the legends. Node size represents significance by converting q-values via log10 transformation and adding to the minimum size of 10 in the case of ageing genes. For gene expression pattern matching, the node size represents significance by multiplying the absolute value of the cosine similarity with 1000. For specific gene class targeting drugs, the seed genes and significant drugs identified via guilt-by-association were connected with each other via shortest path algorithm. For signature reversing or mimicking drugs, all interaction partners of the reversing/mimicking drug were retrieved first and all nodes connected with each other via shortest path algorithm. For unconnected drugs (i.e. no know target genes) the three most similar drugs (based on cosine similarity) were identified and made explicit as edges.

–

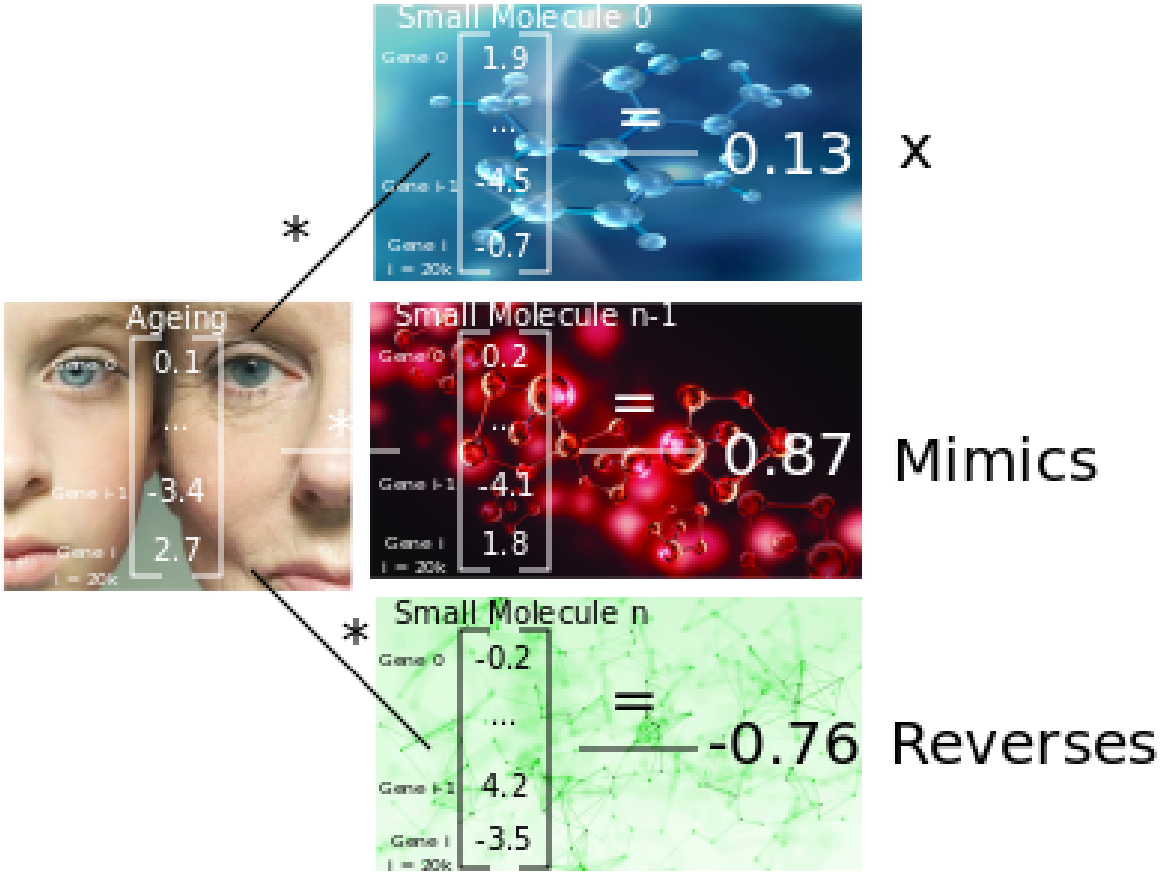

Figure 46: Molecular Signature Matching.

6.3 Results

6.3.1 Ageing Genes Targeting Drugs

Many genes were found to influence lifespan in model organisms as well as in humans [Ageing Genes]. The human genes combined with human homologs of model organisms ageing genes were used as seeds for guilt-by-association to find drugs that target ageing genes [Figure 47 Ageing Gene Targeting Drugs].

Everolimus, Carboplatin, Azd5363, Bmn673 and E7449 are the most significant associations with ageing genes [Figure 47 Ageing Gene Targeting Drugs].

6.3.1.1 Gerontogenes Targeting Drugs

Most drugs inhibit functions. Therefore gerontogenes (a subset of ageing genes that promote ageing) [Ageing Genes] were used as seeds for the guilt-by-association of drugs [Figure 48: Gerontogenes Targeting Drugs].

The most significant drugs targeting gerontogenes are Everolimus, Azd5363, and MK-2206 [Figure 48 Gerontogenes Targeting Drugs].

6.3.1.2 Ageing-Suppressor Genes Targeting Drugs

Certain drugs work via the activation of specific targets. Thus ageing-suppressors genes (a subset of ageing genes that suppress ageing) [Ageing Genes] were utilized for the guilt-by-association [Figure 49 Ageing-Suppressor Targeting Drugs].

Here the most significant drug targeting ageing-suppressors is Carboplatin [Figure 49 Ageing-Suppressor Targeting Drugs].

6.3.1.3 DR Genes Targeting Drugs

Drugs that target dietary restriction-essential genes were identified by using guilt-by-association [Figure 52 Dietary Restriction-Essential Genes Targeting Drugs].

DR-essential genes are most significantly associated with NVP-BGT226, Azd5363, MK-2206, Xl765 and Everolimus [Figure 52 Dietary Restriction-Essential Genes Targeting Drugs].

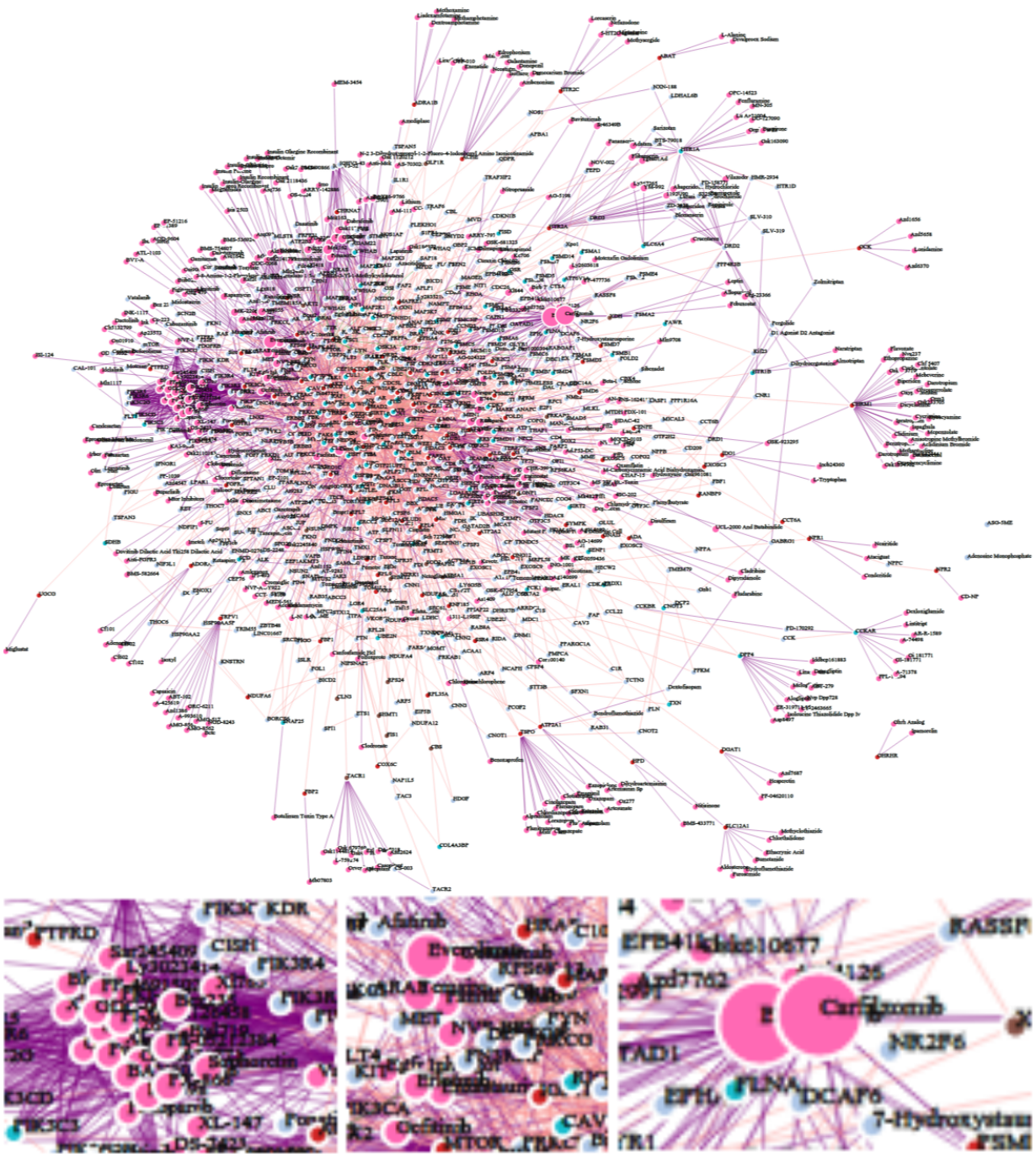

Figure 47: Ageing Gene Targeting Drugs. *Drugs that target ageing genes. Nodes are grey by default, gerontogenes are filled light red, ageing-suppressor genes are filled light blue, ageing genes are purple, and drugs are pink. Physical interactions are pink, genetic interactions are green and drug-protein interactions are purple. Node size represents significance.*

–

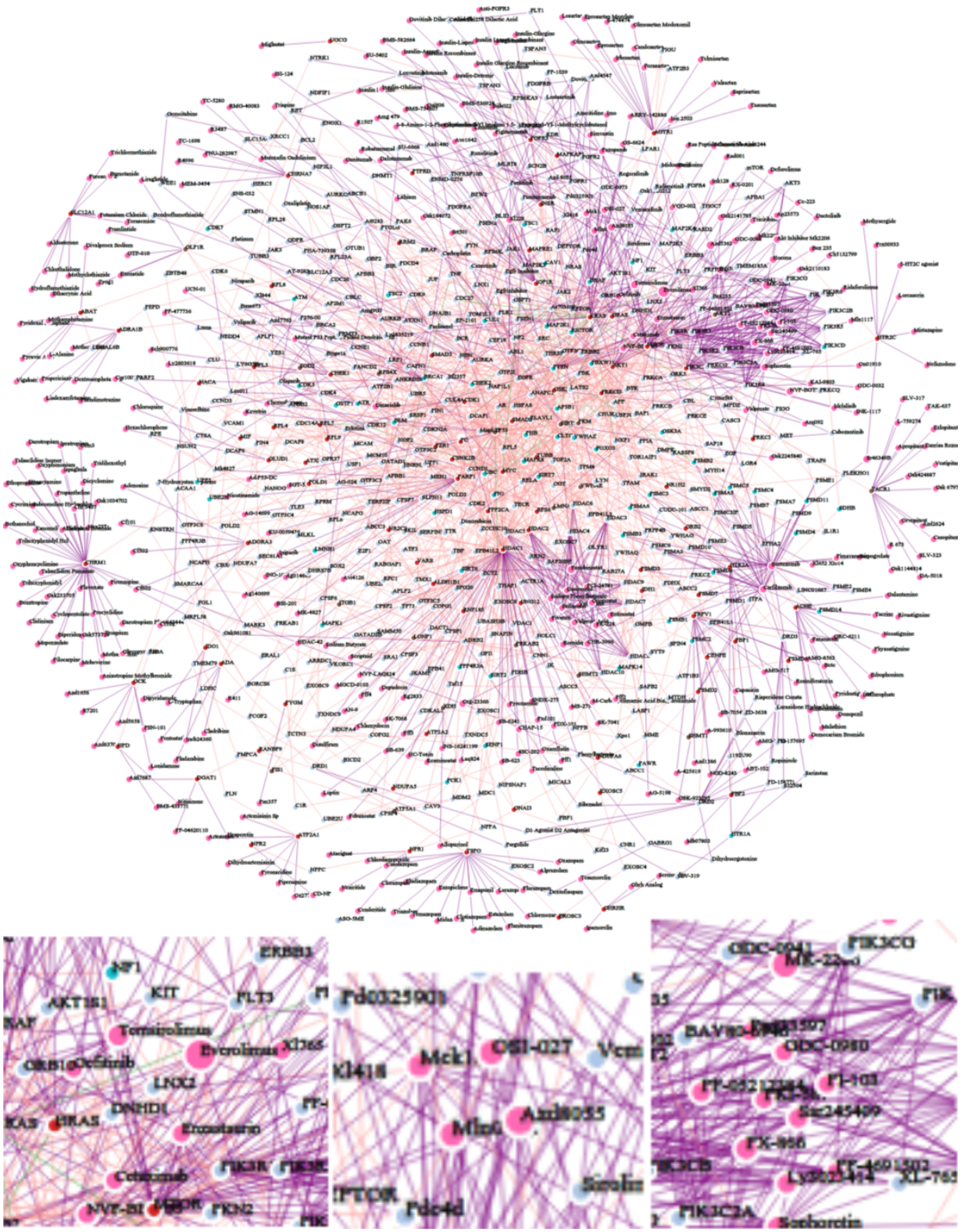

Figure 48: Gerontogenes Targeting Drugs. *Drugs that target gerontogenes were identified via guilt-by-association. Nodes are grey by default, gerontogenes are filled light red, ageing-suppressor genes are filled light blue, ageing genes are purple, and drugs are pink. Physical interactions are pink, genetic interactions are green and drug-protein interactions are purple.*

–

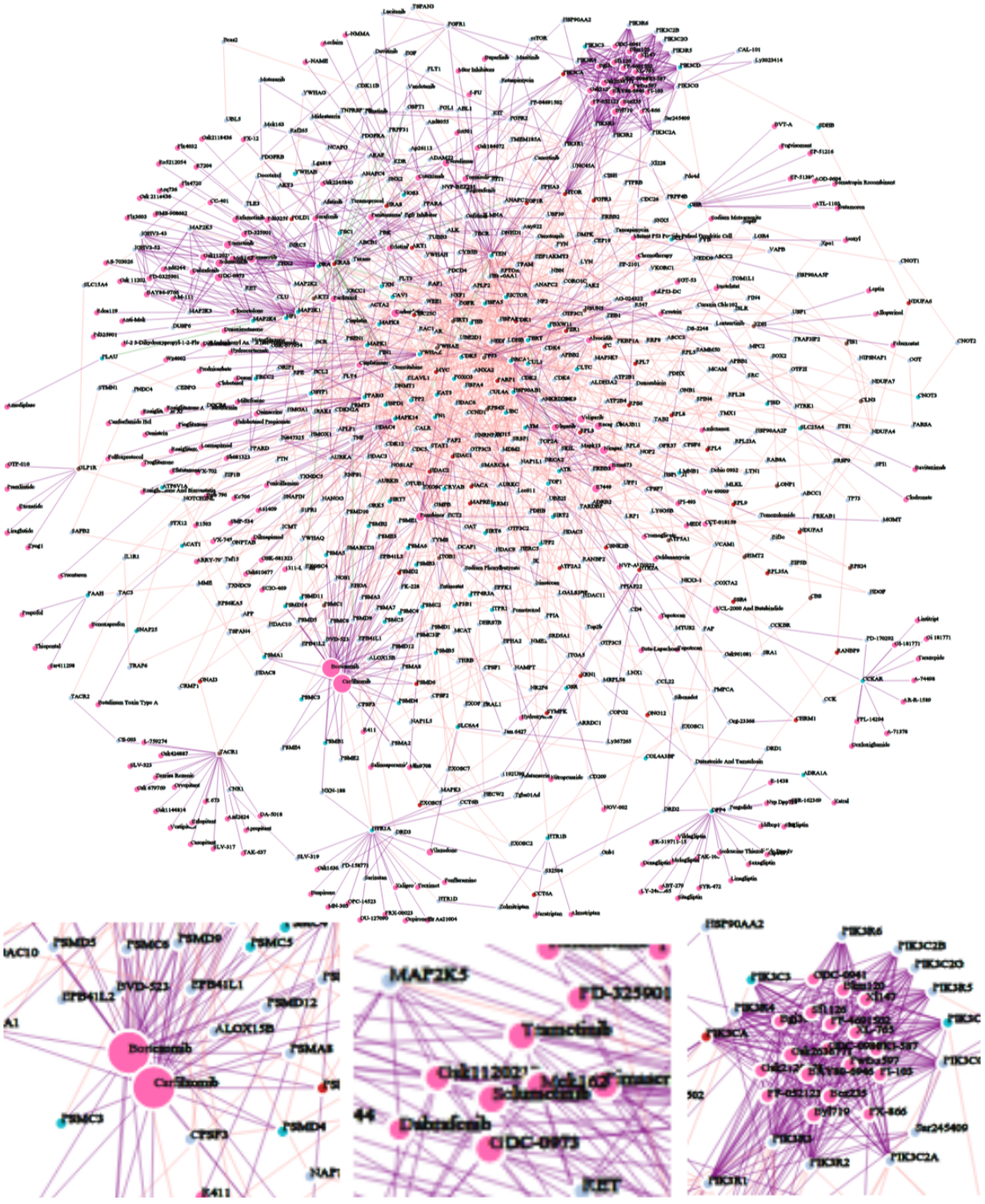

Figure 49: Ageing-Suppressor Genes Targeting Drugs*. Drugs that target ageing-suppressor genes were identified via guilt-by-association. Nodes are grey by default, gerontogenes are filled light red, ageing-suppressor genes are filled light blue, ageing genes are purple, and drugs are pink. Physical interactions are pink, genetic interactions are green and drug-protein interactions are purple. Node size represents significance.*

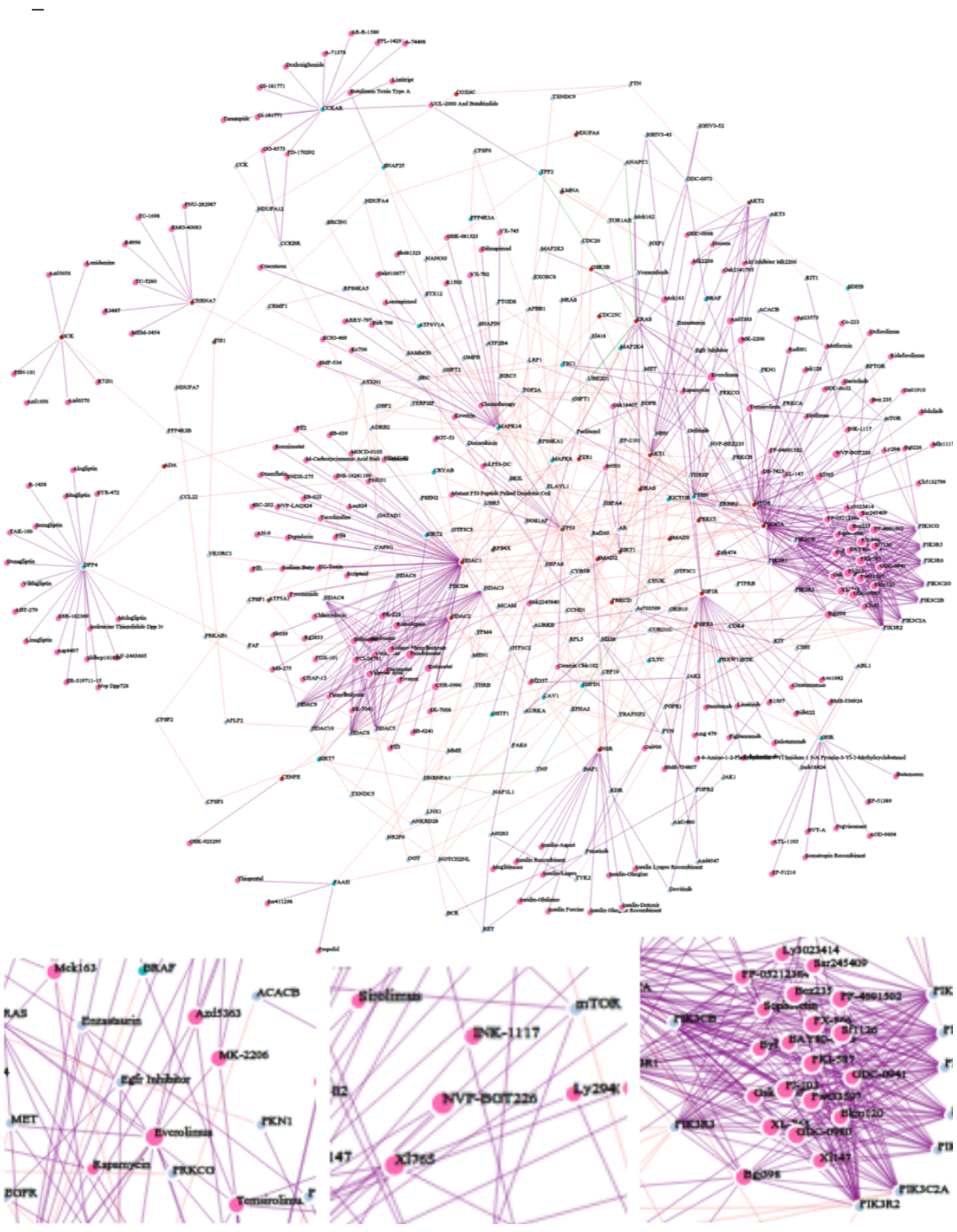

Figure 50: Dietary Restriction-Essential Targeting Drugs*. Drugs that target dietary restriction essential genes were identified via guilt-by-association. Nodes are grey by default, gerontogenes are filled light red, ageing-suppressor genes are filled light blue, ageing genes are purple, and drugs are pink. Physical interactions are pink, genetic interactions are green and drug-protein interactions are purple. Node size represents significance.*

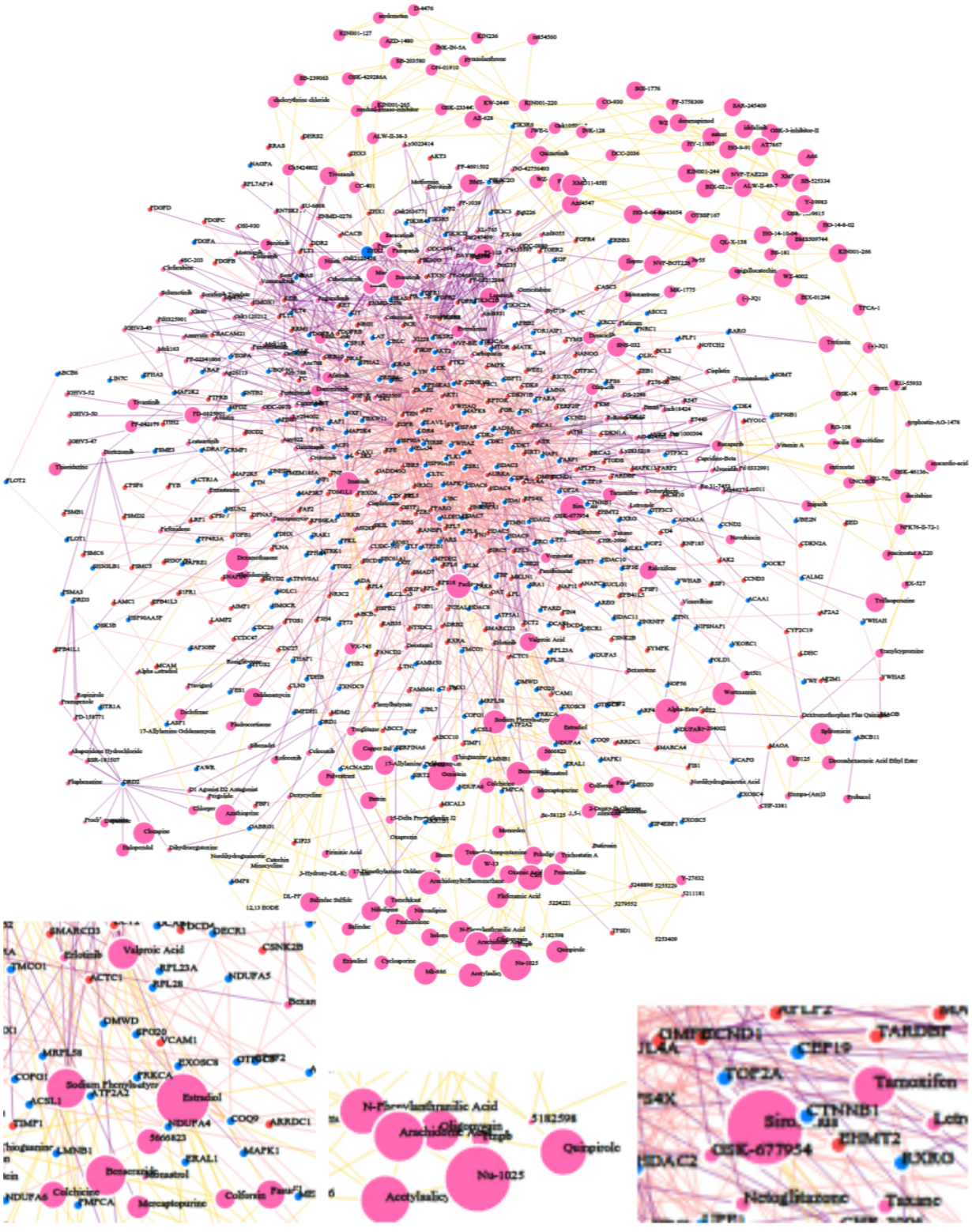

Figure 51: *(******)* ***Ageing Reversing Drugs****. Drugs that reverse ageing gene expression. Nodes are grey by default, upregulated genes are filled light red, downregulated genes are filled light blue, and drugs are pink. Physical interactions are pink, genetic interactions are green, drug-protein interactions are purple, and drug similarity edges are golden. Node size is proportional to the absolute value of the cosinus similarity.*

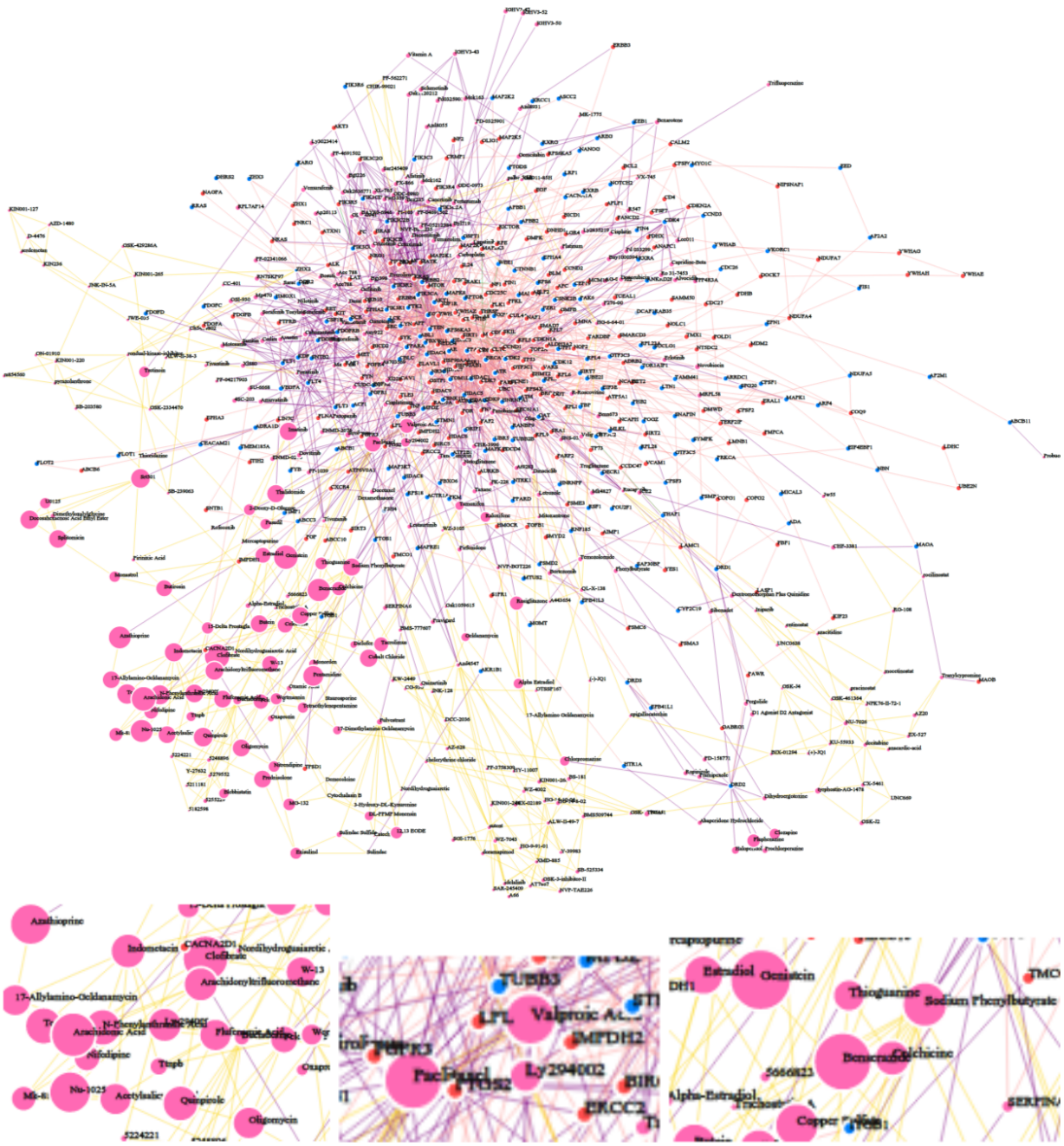

Figure 52: *(††††††)* ***HGPS Reversing Drugs****. Drugs that reverse HGPS gene expression were identified via pattern matching. Nodes are grey by default, upregulated genes are filled light red, downregulated genes are filled light blue, and drugs are pink. Physical interactions are pink, genetic interactions are green, drug-protein interactions are purple, and drug similarity edges are golden. Node size is proportional to the absolute value of the cosinus similarity.*

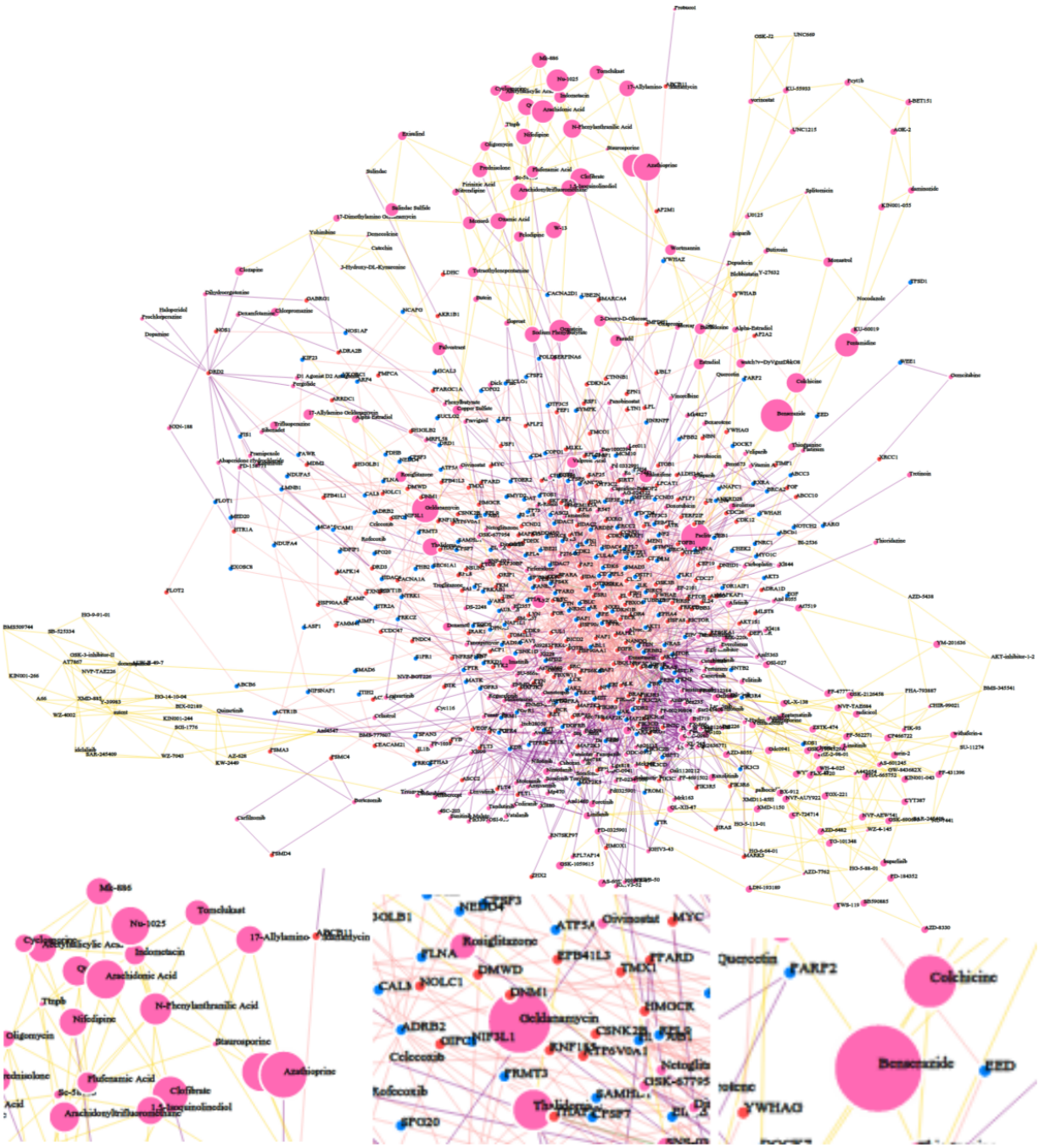

Figure 53: *(‡‡‡‡‡‡)* ***Senescence Reversing Drugs****. Drugs that reverse cellular senescence were identified via pattern matching. Nodes are grey by default, upregulated genes are filled light red, downregulated genes are filled light blue, and drugs are pink. Physical interactions are pink, genetic interactions are green, drug-protein interactions are purple, and drug similarity edges are golden. Node size is proportional to the absolute value of the cosinus similarity.*

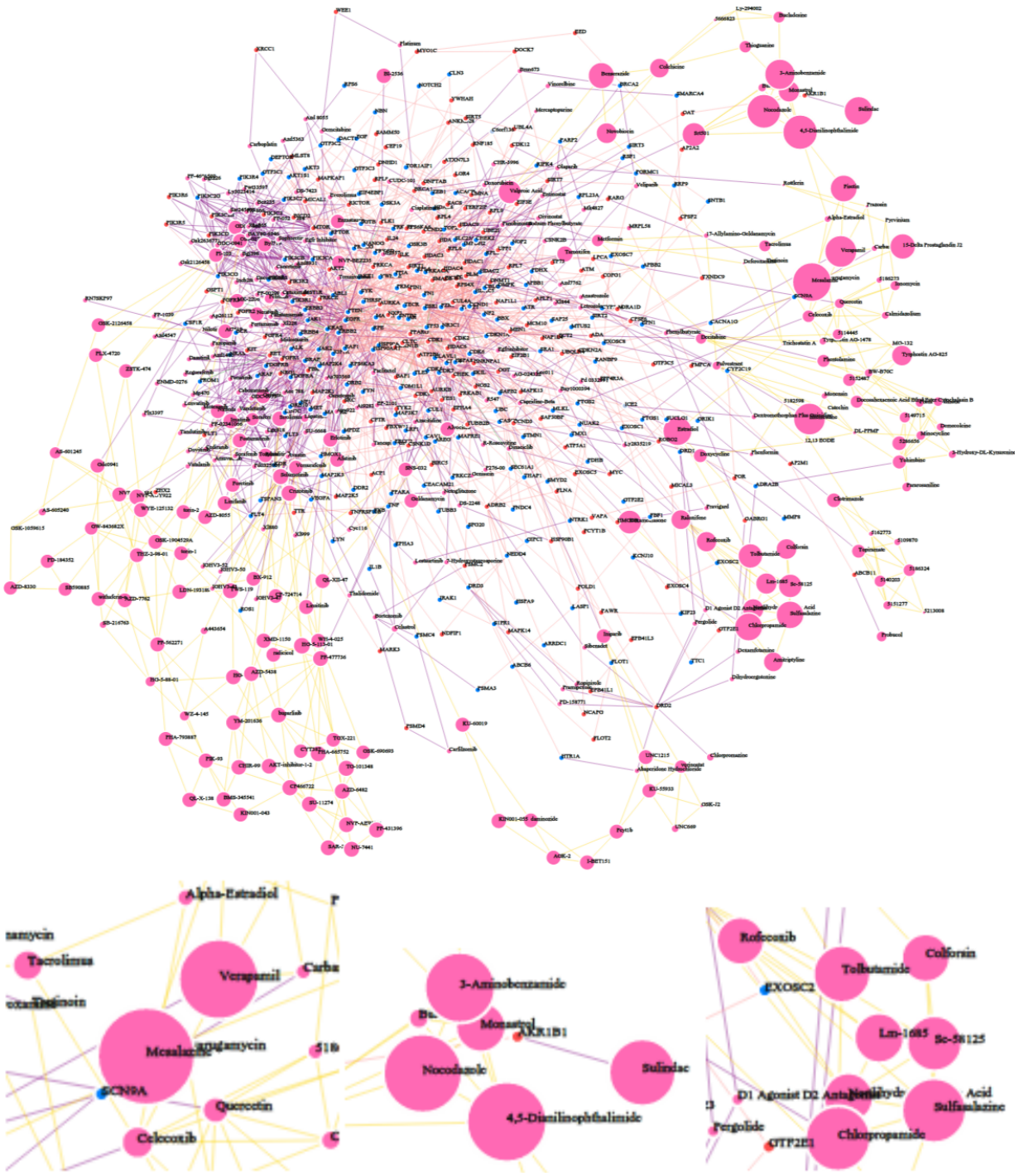

Figure 54: *(§§§§§§)* ***DR Mimetics****. Dietary restriction mimicking drugs were identified via pattern matching. Nodes are grey by default, upregulated genes are filled light red, downregulated genes are filled light blue, and drugs are pink. Physical interactions are pink, genetic interactions are green, drug-protein interactions are purple, and drug similarity edges are golden. Node size is proportional to the absolute value of the cosinus similarity.*

6.3.2 Ageing Reversing Drugs

Reversing the gene expression associated with ageing may reverse the age-related changes and therefore counteract ageing [Ageing Signatures]. Drugs that reverse the tissue-independent common consensus signature of ageing in human were depicted as an interaction network in the following [Figure 47 Ageing Reversing Drugs].

The drugs that reverse the ageing gene expression signatures across tissues the most are Nu-1025, Estradiol, Ly-294002, Sirolimus and Clofibrate [Figure 47 Ageing Reversing Drugs].

6.3.3 HGPS Reversing Drugs

Hutchinson-Gilford progeria syndrome (HGPS) is one of most severe progeroid syndromes that represent accelerated/premature ageing [Ageing Signatures]. Drugs that reverse the consensus signature of HGPS are identified [Figure 52 HGPS Reversing Drugs].

The drugs that reverse the ageing gene expression signatures of HGPS the most are Clofibrate, arachidonic acid, Genistein, Benserazide, and Nu-1025 [Figure 52 HGPS Reversing Drugs].

6.3.4 Senescence Reversing Drugs

Cellular senescence is hallmark of ageing in most mammals. Senescence cells exert negative effects on tissue homeostasis and secrete inflammatory as well as cancer promoting signals [Ageing Signatures]. A consensus signature of senescence is a consensus signature of various forms of cellular senescence such as replicative and stress-induced cellular senescence. Drugs that reverse the consensus signature of senescence were found [Figure 53 Senescence Reversing Drugs].

Azathioprine, Geldanamycin, Pentamidine, Arachidonic Acid, and Ly-294002 are the drugs that reverse the gene expression signatures associated with senescence the most [Figure 53 Senescence Reversing Drugs].

6.3.5 DR Mimetics

Dietary restriction, as the most robust non-genetic intervention that is known to retard ageing across species boundaries, elicits a transcriptional signature that interferes with the ageing process. Drugs that mimic the effect of dietary restriction based on the contribution of each individual gene within a signature were found and depicted in the following manner [Figure 54 DR Mimetics].

Mesalazine, 4,5-Dianilinophthalimide, Nocodazole, Verapamil (Dexverapamil), 3-Aminobenzamide are the drugs with the most similar gene expression profile to dietary restriction [Figure 54 DR Mimetics].

6.4 Discussion

Via graph theory and gene expression pattern matching, drug candidates were identified that target ageing genes, specific subclasses of ageing genes and dietary restriction-essential genes as well as potentially reverse ageing, HGPS, senescence and mimic dietary restriction at the level of the transcriptome.

Several drugs were found to be shared among the results of these different investigations. It was also noted that many small molecules that are known to extend lifespan were among the results such as sirolimus. Sirolimus, also known as rapamycin, is a macrolide immunosuppressant (Streit, et al., 1996). Sirolimus was found to target ageing and DR-essential genes, reverse ageing, mimic DR, reverse HGPS and has an effect on reversing cellular senescence [Ageing Genes Targeting Drugs; DR Genes Targeting Drugs; Ageing Reversing Drugs; DR Mimetics; HGPS Reversing Drugs; Senescence Reversing Drugs]. Therefore rapamycin (sirolimus) was re-identified to mimic the effect of DR and reverse the gene expression patterns associated with ageing. However, other drugs were found that are higher ranked than rapamycin and represent potential candidates for drugs that are similar or more effective than rapamycin.

Everolimus is a rapamycin derivative which is an allosteric mTOR inhibitor (Raimondo, et al., 2016). Everolimus reverses ageing and HGPS as well as targets ageing genes (gerontogenes and

ageing-suppressors) and DR-essential genes [Ageing Reversing Drugs; HGPS Reversing Drugs; Ageing Genes Targeting Drugs; Gerontogenes Targeting Drugs; Ageing-Suppressor Genes Targeting Drugs].

Carboplatin is a platinum containing anticancer drug (Jangir & Mehrotra, 2014). A combination therapy with Carboplatin is expected to result in life extension of cancer patients (Sato, et al., 2006). Carboplatin targets ageing genes, more specifically ageing-suppressor genes [Ageing-Suppressor Genes Targeting Drugs].

AZD5363, an inhibitor of protein kinase B (AKT) and affects PI3K/mTOR (Choi, et al., 2016). AZD5363 targets ageing genes, but more specifically gerontogenes and DR-essential genes [Gerontogenes Targeting Drugs; DR Genes Targeting Drugs].

BMN673 is a PARP inhibitor (Dai, et al., 2016). BMN673 targets ageing, specifically ageing-suppressor genes [Ageing-Suppressor Genes Targeting Drugs]. E7449 is a potent PARP1/2 inhibitor that also inhibits PARP5a/5b (McGonigle, et al., 2015). E7449 similar to BMN673 specifically targets gerontogenes [Gerontogenes Targeting Drugs].

NU-1025 is a PARP-1 inhibitor (Li & Osborne, 2008) that exhibits neuroprotective activity (Kaundal, et al., 2006). NU1025 conveys neuroprotection associated with reversal of NAD depletion and reduction in DNA fragmentation (Kaundal, et al., 2006). Nu-1025 reverses ageing, senescence and HGPS [Ageing Reversing Drugs; HGPS Reversing Drugs; Senescence Reversing Drugs]. It might not work via the DR mechanism.

Estradiol is a steroid hormone (Botelho, et al., 2009) and the primary female sex hormone (Stoeva, 1989). Estradiol reverses ageing, HGPS and senescence as well as mimics DR [Ageing Reversing Drugs; DR Mimetics; HGPS Reversing Drugs; Senescence Reversing Drugs]. However, estradiol is a potent carcinogen in postmenopausal women.

LY-294002 is a phosphoinositide 3-kinase (PI3K) inhibitor (McDowell, et al., 2004). LY-294002 reverses ageing, HGPS and senescence in addition to mimic DR [Ageing Reversing Drugs; DR Mimetics; HGPS Reversing Drugs; Senescence Reversing Drugs].

Clofibrate is an established PPARalpha ligand (Wang, et al., 2013) that is used as antidiuretic drug (Tarpey & Mendoza, 1980) and has anticancer activity (Wang, et al., 2013). Clofibrate reverses ageing, HGPS and senescence [Ageing Reversing Drugs; HGPS Reversing Drugs; Senescence Reversing Drugs].

Arachidonic acid is a precursor of prostaglandins, is released by phospholipase A2 (Yoshida, et al., 2007). Arachidonic acid reverses ageing, HGPS and senescence [Ageing Reversing Drugs; HGPS Reversing Drugs; Senescence Reversing Drugs].

Genistein is a phytoestrogen that has estrogenic activity (Kwon, et al., 2007). Genistein interacts with oestrogen receptors alpha and beta and elicits reproductive effects in developing rodents (Soucy, et al., 2006). Genistein reverses ageing, HGPS and senescence while also mimicking DR [Ageing Reversing Drugs; DR Mimetics; HGPS Reversing Drugs; Senescence Reversing Drugs].

Benserazide is commonly used for Parkinson's disease, which is an age-related neurodegenerative condition. It is used in combination with L-DOPA as a peripheral aromatic L-amino acid decarboxylase inhibitor. Benserazide acts also in the central nervous system (Shen, et al., 2003). Benserazide reveres ageing, HGPS, and senescence as well as mimics DR [Ageing Reversing Drugs; DR Mimetics; HGPS Reversing Drugs; Senescence Reversing Drugs].

Azathioprine is used as an anti-inflammatory agent (Moeslinger, et al., 2006). Azathioprine reverses ageing, HGPS and senescence [Ageing Reversing Drugs; HGPS Reversing Drugs; Senescence Reversing Drugs].

Geldanamycin is a ligand of the heat shock protein 90 (Li, et al., 2012). Geldanamycin reveres ageing, HGPS, and senescence as well as mimics DR [Ageing Reversing Drugs; DR Mimetics; HGPS Reversing Drugs; Senescence Reversing Drugs].

Pentamidine is a antiprotozoal agent used for the treatment of Pneumocystis jirovecii pneumonia (Arino, et al., 2012). Pentamidine reverses ageing, HGPS and senescence [Ageing Reversing Drugs; HGPS Reversing Drugs; Senescence Reversing Drugs].

MK-2206 is a allosteric Akt inhibitor that also inhibits tumour growth (Sangai, et al., 2012). MK-2206 mimics DR and reverses ageing as well as targets ageing genes, specifically DR-essential genes and gerontogenes [DR Mimetics; Ageing Reversing Drugs; DR Genes Targeting Drugs; Gerontogenes Targeting Drugs].

NVP-BGT226 is a dual inhibitor of PI3K and mTOR (Fokas, et al., 2012). NVP-BGT226 reverses ageing, HGPS and senescence as well as targets ageing genes, specifically gerontogenes and DR-essential genes [Ageing Reversing Drugs; HGPS Reversing Drugs; Senescence Reversing Drugs; Gerontogenes Targeting Drugs; DR Genes Targeting Drugs].

XL765, also known as SAR245409, is a potent and highly selective pan inhibitor of class PI3Ks (alpha, beta, gamma, and delta) with activity against mTOR (Yu, et al., 2014). XL765 targets ageing genes, including gerontogenes and DR-essential genes [Gerontogenes Targeting Drugs; DR Genes Targeting Drugs].

Mesalazine is a standard first line treatment for moderately active ulcerative colitis (Buckland & Bodger, 2008). Mesalazine mimics DR [DR Mimetics].

4,5-Dianilinophthalimide is a EGF family tyrosine kinase receptor inhibitor (Yen & Soong, 1996). It is considered for use in treatment of Alzheimer's disease, an age-related neurodegenerative disease (Hennessy & Buchwald, 2005). 4,5-Dianilinophthalimide mimics DR [DR Mimetics].

Nocodazole is a synthetic anti-tubulin agent (Samson, et al., 1979) used as anticancer drug (Attia, et al., 2015). Nocodazole mimics DR and reverses senescence [DR Mimetics; Senescence Reversing Drugs].

Dexverapamil is a competitive inhibitor of the P-glycoprotein efflux pump (Wilson, et al., 1995). Dexverapamil mimics DR [DR Mimetics];

3-Aminobenzamide is a potent inhibitor of poly(ADP-ribose)synthetase (Masiello, et al., 1985). 3-Aminobenzamide mimics DR [DR Mimetics].

Other known geroprotectors like metformin (Bulterijs, 2011) target specifically gerontogenes, reverse ageing and mimic DR [DR Mimetics]. Metformin extends the lifespan of model organisms from invertebrates to rodents (Anisimov, et al., 2011; Mair, et al., 2011).

Quercetin is a flavonoid that acts as an antioxidant and occurs in foods mainly as glycosides (Olthof, et al., 2000). Quercetin is a well known senolytic. Here it was found that quercetin mimics DR and reverses senescence (Malavolta, et al., 2016) [DR Mimetics; Senescence Reversing Drugs].

The application of wortmannin, a PI3K inhibitor, could extend the lifespan (MacKay, et al., 2012). Wortmannin reverses ageing, senescence and progeria [Ageing Reversing Drugs; Senescence Reversing Drugs; HGPS Reversing Drugs].

Trichostatin A (TSA) is a potent and specific histone deacetylase inhibitor with promising antitumour activity (Sanderson, et al., 2004). Trichostatin A was found to extend the lifespan of *D. melanogaster* by promoting *hsp22* gene transcription, and affecting the chromatin morphology at the locus of the hsp22 gene (Tao, et al., 2004). TSA increases lifespan in both long- and short-lived fruit fly lines, with variable degrees (up to 25%) (Zhao, et al., 2005). Among the small molecules that reverse premature ageing was trichostatin A. Trichostatin A among other compounds were found to have the potential to also mimic the gene expression changes induced by dietary restriction. Trichostatin A reverses ageing and HGPS [Ageing Reversing Drugs; HGPS Reversing Drugs].

In the network of dietary restriction-essential genes there is a cluster of drugs that target histone deacetylases [Figure 50 Dietary Restriction-Essential Genes Targeting Drugs]. Caloric restriction induces a hyperactylation state, thus it may work via inhibiting protein deacetylases. This links to lipoic acid given as dietary supplement to extend group survival (Merry, et al., 2008).

Several drugs could have not been found via interactomics alone as there are is no obvious enrichment for ageing genes. The utilization of gene expression data enables to find drugs for which there are even no known target genes or interactions.

There seem to be at least two categories of drugs; those that work via DR or those that counteract ageing, HGPS and senescence. This is similar to what was observed regarding genes in respect to DR. Some genes (DR essential) if perturbed affect lifespan, i.e. interferes with ageing via the DR mechanism while others do not affect lifespan but do not work via the DR pathways.

Drug combination could be predicated as well. Those could be more powerful in reversing ageing-related gene expression changes completely than single compounds are capable of.

6.5 Conclusion

Graph theory was used to find small molecules that target specific ageing gene classes such as gerontogenes and ageing-suppressor genes as well as DR-essential genes. Further gene expression matching based on calculation of the cosine similarity/distance was utilized to find drugs that reverse or mimic the molecular signatures related to ageing, HGPS, cellular senescence and dietary restriction. Previous known geroprotectors were rediscovered and put into context while novel small molecules with potential geroprotector activity were predicted. Similar to what was discovered in genetic experiments it appears that small molecule that affect the lifespan are of two kind: Those that work via DR signalling and those that do not.

7 Discussion

This section is a critical examination of the findings in the light of the previous state of the subject as outlined in the background, and makes judgements as to new insights gained from the work. Overall, this work shows that it is possible to make predictions about ageing and interventions to retard ageing from omics data.

The introduction defined what is ageing, age and ageing biomarkers as well as interventions to intervene with ageing such as dietary restriction (DR) and pharmacologically with anti-ageing drugs. In the following various interrelated disciplines involving the use of computer science such as computational biology, bioinformatics, functional genomics, and systems biology have been explained and described how they relate to each other [Figure 1 Overlapping Fields] and lastly the important concepts of molecular profiling and signatures has been defined (Wuttke & de Magalhaes, 2011).

The approach undertaken starts from the genome by classifying those genes that are associated with ageing (inferred from gene manipulation experiments) and applied graph theory, to biological networks in order to characterize those genes already known to be associated with ageing and predict novel ageing genes, not yet implicated in the ageing process. Ageing genes can be subdivided into the defined classes of gerontogenes and ageing-suppressor genes.

Following that analysis other omics data such as transcriptomics are explored and molecular signatures of ageing have been derived in order to investigate gene activities. A particular class of ageing genes that are necessary for the lifespan extending effect of dietary restriction, so called DR-essential genes, is defined and used as an indicator for seeking novel interventions. Subsequently molecular signatures of dietary restriction were derived and characterized. Next, drugs that target ageing genes and specific subclasses of ageing genes have been identified. Signatures of small molecules have been derived and used to identify (via pattern matching) anti-ageing compounds that are highly likely to interfere with the ageing process, perhaps more effectively than previous interventions.

7.1 Genes that Govern Ageing

In this thesis ageing genes (sometimes also referred to as longevity genes) have been subclassified into gerontogenes and ageing-suppressor genes. Another class of ageing-associated genes, called DR-essential genes (or DR genes for short) have been defined as well. Further a class of genes that were found to be associated with exceptional longevity, i.e. longevity-associated genes and cellular senescence were proposed. In the following is the taxonomy used in this study:

- Ageing-Associated Gene
    - Ageing Gene (a.k.a. Longevity Gene or Longevity Assurance Gene or Longevity/Ageing-Regulating Gene)
        - Gerontogene (a.k.a. Anti-Longevity/Pro-Ageing Gene)
            - Genuine Gerontogene
            - Uncertain Gerontogene
        - Ageing-Suppressor Gene (a.k.a. Pro-Longevity/Anti-Ageing Gene)
            - Genuine Ageing-Suppressor Gene
            - Uncertain Ageing-Suppressor Gene
    - DR Gene (a.k.a. DR-Essential Gene)
    - Longevity-Associated Gene
    - Cellular Senescence Gene

Ageing genes and DR-essential genes have been identified by gain- and loss-of-function genetic experiments. Ageing-differentially expressed and longevity-associated genes have been primarily

identified via measuring transcript levels and genotyping genomes respectively (both via either low-throughput or high-throughput experiments). Many longevity regulating pathways reveal anti-ageing and ageing-promoting effects and therefore change lifespan, depending on the biological and environmental context (Honjoh & Nishida, 2011). Thus, the distinction into those defined classes might not always be so clear cut. Those cases where an ageing gene can both act as a genuine gerontogene and a genuine ageing-suppressor gene or has no effect in different contexts is challenging this simplification.

As age-related diseases are caused by ageing it is not surprising that mutations that slow down ageing also postpone age-related diseases (Kenyon, 2010). Another class to be considered are the age-related disease genes. It would be intriguing to know to what extent age-related disease genes and ageing genes overlap and whether either one interacts with the other. Genes associated with age-related disease were shown to be informative as providing prior knowledge for identifying longevity-associated genes (Fortney, et al., 2015).

Age-related disease and normal ageing are often differentiated and assumed to be governed by separate molecular mechanisms. Molecular profiling may substantiate or reject this proposal (Pereira, et al., 2007). In this work, drugs predicted to intervene with ageing were associated with the treatment of several age-related diseases including neurodegeneration and cancer [Small Molecule Predictions].

Mutations that extend the lifespan also frequently prevent tumour cell growth (Pinkston, et al., 2006). It would be interesting to investigate how far is the parallel between gerontogenes/ageing-suppressor genes and proto-oncogene/tumour-suppressor genes. Are gerontogenes more likely to be proto-oncogenes while ageing-suppressor genes tend to be tumour-suppressor genes or vice versa or is their relationship non-trivial if there is any?

Ageing genes frequently interact with multiple signalling and metabolic pathways (Curtis, et al., 2006). Here DR-essential genes in particular but also ageing genes were found to have a higher node degree than expected by chance, even higher than signalling genes.

Several genetic pathways regulate organism longevity and act by modifying gene expression. Many more genes are subjected to age-associated transcriptional regulation. Dietary restriction is capable of preventing some age-related gene expression changes. Manipulating the expression of some age-related genes can robustly extend an organism's lifespan. Homologous genes in other organisms tend to have similar functions. The activity of many transcription regulatory elements are linked to biological age as opposed to chronological age, suggesting that orderly and tightly controlled regulatory pathways are active during ageing. An intricate program of gene expression controls progression through the different stages in development. Ageing is genetically determined and environmentally modulated (Seroude, 2002). The finding of this research here on ageing genes and consensus molecular transcriptional signatures found repeatedly growth and development tightly linked to ageing. In particular gerontogenes are positive regulators of growth while ageing-suppressors are negative regulators [Ageing Genes].

The results of these predictions regarding DR-essential genes were reproduced and validated in other studies (Kitamura, et al., 2006; Bohnert & Kenyon, 2017). This further and independently confirms the validity of these predictions.

7.2 Age-Related Changes

There are myriads of age-related changes continuously happening in organisms. Those kind of changes can be classified into discrete categories. There have been attempts to define those classes of age-related changes, but none is comprehensive enough to include all kinds of age-related change (Lopez-Otin, et al., 2013). First of all changes are happening on different scales. There are changes happening at the level of the whole physiology down to the molecular level. To classify the different scales the following taxonomy is used:

- Physiological Change
- Organ-Level Change
- Tissue-Level Change
- Cellular Change

- Organelle-Level Change
- Molecular Change

With the interactome and transcriptome as well as most other omics data the entities are only acting at the molecular level of genes, transcripts, protein/RNA products and small molecules (including metabolites). However, via the shared associations it is possible to obtain some insights into the changes at the level of the other scales as they are composed hierarchically of those molecular building blocks.

Sometimes these changes are called "damages", but this is often misleading as those changes are not all damaging. Damage is something very subjective. It might be a correct description for certain changes at the molecular level such as DNA damage, but those are just resulting into mutations and sometimes required for the immune system to generate antibody variety, during recombination of genomes in meiosis and for evolution. In addition, changes that are happening to proteins can be oversimplified and just attributed as damages (though they are often just modifications). However, cells use those modifications as ways of signalling for example regarding oxidation and glycation of proteins. Overall it should be avoided to attribute every age-related change as damage.

1. Genomic Instability (Molecular)
    - Nuclear DNA Mutation (Molecular)
    - Telomere Attrition (Molecular)
    - Mitochondrial DNA Mutation (Molecular)
2. Epigenetic Alteration (Molecular)
3. Transcript/Protein Decoupling (Cellular)
4. Proteostasis Loss (Cellular)
    - i.e. Loss of Protein Homeostasis
    - Lysosomal Dysfunction (Organelle-level)
    - Proteasome Dysfunction (Molecular)
5. Nutrient Sensing Deregulation (Cellular)
6. Signalling Alteration (Molecular)
7. Mitochondrial Dysfunction (Organelle-level)
8. Intercellular Communication Alteration (Physiological)
    - Immunosenescence (Physiological)
9. Cellular Senescence (Cellular)
10. Stem Cell Exhaustion (Physiological)
11. Cycle Alteration (Molecular - Physiological)

7.3 Genomic Instability

7.3.1 Nuclear DNA

7.3.1.1 DNA Repair

Ageing genes are involved in DNA repair and in particular ageing-suppressor genes are involved in response to DNA damage and various DNA repair forms [Figure 10 Common Ageing Gene Network]. Genes differentially expressed during ageing are also involved in DNA repair [Ageing Signatures]. Genes upregulated during ageing are commonly associated to the response to DNA damage [Ageing Signatures; Figure 21 Common Ageing], while genes downregulated with ageing are associated with positive regulation of DNA repair. DNA repair is also downregulated in cellular senescence [Ageing Signatures].

DR-essential genes are involved in regulation of DNA repair, DNA double-strand break repair, positive regulation of DNA repair, UV-damage excision repair, DNA synthesis involved in DNA repair, pyrimidine dimer repair by nucleotide-excision repair, single strand break repair [Dietary Restriction Genes]. Therefore DR-essential genes are involved in DNA repair and its positive regulation.

Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved in DNA repair, DNA double-strand break repair, double-strand break repair via homologous recombination, and recruitment and ATM-mediated phosphorylation of repair and signalling proteins at DNA double strand breaks. Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly involved in double-strand break repair, nucleotide excision repair, base excision repair, and nucleotide-excision repair DNA damage recognition. Gerontogene are commonly involved in global genome nucleotide excision repair GG-NER. Ageing-suppressor genes are involved in DNA repair complex, TP53 regulates transcription of DNA repair genes, double-strand break repair via nonhomologous end joining, transcription-coupled nucleotide excision repair TC-NER, DNA synthesis involved in DNA repair, gap-filling DNA repair synthesis and ligation in TC-NER, mismatch repair, mismatch repair (MMR) directed by MSH2:MSH3 MutSbeta, nucleotide-excision repair factor 1 complex, meiotic mismatch repair, nucleotide-excision repair complex, base-excision repair, mismatch repair complex, SUMOylation of DNA damage response and repair proteins, mismatch repair (MMR) directed by MSH2:MSH6 MutSalpha, nucleotide excision repair, and homology directed repair [Ageing Genes].

Genes differentially expressed during ageing and upon DR are commonly involved in DNA repair [Ageing Signatures; Dietary Restriction Signatures]. Gap-filling DNA repair synthesis and ligation in TC-NER is downregulated upon DR. Though DR-downregulated genes were associated with double-strand break repair via non-homologous end joining in humans, which may mean that there are fewer DNA double-strand breaks occurring under DR, or/and it is downregulated in favour of the more exact homologous repair mechanism. Regardless, DR is associated with reduced DNA mutations [Dietary Restriction Signatures]. DR-essential genes are involved in regulation of DNA repair, positive regulation of DNA repair, DNA synthesis involved in DNA repair DNA double-strand break repair, recruitment and ATM-mediated phosphorylation of repair and signalling proteins at DNA double strand breaks, single strand break repair, UV-damage excision repair, and pyrimidine dimer repair by nucleotide-excision repair [Dietary Restriction Genes].

Cellular senescence downregulates DNA repair, DNA synthesis involved in DNA repair, and regulation of DNA repair. Genes upregulated in cellular senescence are involved in negative regulation of DNA repair [Ageing Signatures].

DNA repair is mostly associated with ageing-suppressor genes, and differentially expressed during ageing and under DR conditions. Cellular senescence suppresses the repair of DNA, which may lead to enhanced DNA damage in senescence cells and a negative feedback loop that locks senescence cells down.

7.3.1.2 Telomere Attrition

Ageing genes are involved in telomere maintenance. Gerontogenes negatively regulate telomere maintenance, while ageing-suppressor genes positively regulate telomere maintenance and telomerase activity [Ageing Genes]. In particular ageing-suppressor genes are commonly associated with participating in telomere maintenance (including telomere maintenance via recombination) and being located in the nuclear chromosome telomeric region [Figure 10 Common Ageing Gene Network]. Ageing is observed to downregulate telomere maintenance and the positive regulation of telomerase maintenance [Ageing Genes]. DR downregulates senescence induced by DNA damage or telomere stress [Ageing Signatures].

Additionally, positive regulation of telomerase activity is significantly and commonly associated with downregulation during ageing [Ageing Signatures]. More specifically ageing-differentially expressed genes are involved in positive regulation of establishment of protein localization to telomere [Figure 17 M. musculus Ageing], while ageing-downregulated genes are involved in positive regulation of telomere maintenance via telomerase as well as telomere tethering at the nuclear periphery [Figure 17 M. musculus Ageing; Figure 20 S. cerevisiae Ageing]. TERT itself is downregulated in the human consensus signature. Telomere capping and telomere organization are associated with DR-downregulated genes in humans that may reflect a lower level of repair required for the well-maintained telomeres under dietary restriction

conditions [Figure 39 H. sapiens Dietary Restriction]. Telomere shortening is happening during normal ageing as observed in both human and mice (Blasco, 2007). Dietary restriction of amino acids other than methionine increases telomerase activity and prevents telomere shortening in rats (Tanrikulu-Kucuk & Ademoglu, 2012). Dietary restriction can also enhance telomere maintenance without increased telomerase activity (Wang, et al., 2010), probably via recombination.

7.3.1.3 Nuclear Envelope

The nuclear envelope is a double lipid bilayer enclosing the nucleus.

Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly located in the nuclear envelope. Ageing-suppressor genes are involved in nuclear envelope organization, nuclear envelope reassembly, and initiation of nuclear envelope reformation [Ageing Genes].

Genes downregulated during ageing are commonly located in nuclear envelope and involved in nuclear envelope breakdown. The nuclear envelope and nuclear envelope organization is significantly associated with ageing-upregulated genes [Figure 13 H. sapiens Ageing].

DR-essential genes are involved in nuclear envelope organization [Dietary Restriction Genes]. Genes differentially expressed (both up- and down-regulated) during DR are located in the nuclear envelope. Genes upregulated upon DR are involved in nuclear envelope breakdown. Genes downregulated upon DR are involved in nuclear envelope organization, nuclear envelope reassembly, and initiation of nuclear envelope reformation [Dietary Restriction Signatures].

Ageing genes are located in the nuclear pore and involved in nuclear pore organization. More specifically ageing-suppressor genes are located in the nuclear pore inner ring and make up structural constituent of nuclear pore [Ageing Genes]. Genes downregulated with ageing are involved in nuclear pore organization as well as are located in nuclear pore, structural constituent of nuclear pore, nuclear pore central transport channel, and nuclear pore cytoplasmic filaments. Cellular senescence downregulated genes are involved in nuclear pore complex disassembly [Ageing Signatures].

Ageing-suppressor genes are majorly involved in nuclear envelope and there are changes happening in the expression of genes involved in the nuclear envelope both during ageing and upon DR. Ageing downregulates nuclear envelope breakdown, while DR upregulates this process. Upregulation of nuclear envelope breakdown might enable the replacement of long-lived proteins associated with the nuclear envelope such as nuclear pore complexes. Nuclear envelope alterations occur during physiological and premature ageing. The resulting nuclear envelope defects are causal for stem cell dysfunctions (Espada, et al., 2008).

7.3.1.4 Chromosomes

Ageing genes are involved in chromosome organization and regulation of chromosome organization. Gerontogenes are involved in negative regulation of chromosome organization. Ageing-suppressor genes are also significantly associated with being involved in chromosome organization and regulation of chromosome organization. Ageing-suppressor genes are associated with chromosome centromeric region, condensed chromosome, chromosome segregation, and chromosome organization involved in meiotic cell cycle [Ageing Genes]. Genes downregulated with ageing are involved in chromosome organization and regulation of chromosome organization and positive regulation of chromosome organization. Cellular senescence downregulates genes that are involved in chromatin organization [Ageing Signatures]. DR upregulates chromosome organization while downregulating nuclear chromosome [Dietary Restriction Signatures].

Chromosome organization appears to be an ageing-suppressing process that is downregulated during ageing and in cellular senescence, but upregulated upon DR.

7.3.2 Mitochondrial DNA

Gerontogenes and ageing-suppressors are involved mitochondrial genome maintenance. Gerontogene are commonly involved in mitochondrial DNA repair and replication. Ageing-suppressor genes are also involved in mitochondrial DNA repair and replication [Ageing Genes]. Genes upregulated upon DR are involved in regulation of mitochondrial DNA metabolic process [Ageing Signatures].

Therefore, it seems like that DNA repair involves ageing-suppressor genes, is downregulated during ageing, and might be modulated by DR via positive regulation. Similar ageing-suppressor genes promote telomere maintenance, while gerontogenes counteract maintenance of telomerase. Ageing downregulates telomere maintenance.

7.4 Epigenetic Alterations

Epigenetic changes involve DNA methylation and hydroxymethylation as well as post-translational modifications of histones such as methylation, acetylation, ubiquitination, and phosphorylation among others and chromatin remodelling. Epigenetic modifications vary greatly over the course of development and ageing, but are remarkable consistent between individuals and between mice and humans.

7.4.1 Chromatin

Ageing genes are commonly involved in chromatin silencing and particular chromatin silencing at rDNA. Ageing genes including gerontogenes and ageing-suppressor genes are commonly involved in chromatin and chromatin organization as well as covalent chromatin modification. Ageing-suppressor genes are involved in chromatin binding regulation of chromatin organization and negative regulation of chromatin organization [Ageing Genes]. Ageing-suppressor genes are commonly associated with chromatin including nuclear chromatin, chromatin binding and NAD-dependent histone deacetylase activity [Figure 10 Common Ageing Gene Network]. Ageing-suppressor genes are associated with chromatin silencing at rDNA nuclear telomeric heterochromatin and nuclear telomeric heterochromatin chromatin silencing at silent mating-type cassette [Figure 8 S. cerevisiae Ageing Gene Network]. Gerontogenes also exhibit chromatin binding (Figure 5 M. musculus Ageing Gene Network). Overall ageing genes are commonly associated with chromatin, chromatin binding, covalent chromatin modification, regulation of chromatin organization and histone modifications [Figure 10: Common Ageing Gene Network].

Genes differentially expressed with ageing are commonly involved in chromatin, chromatin binding, covalent chromatin modification and chromatin organization. Genes downregulated with ageing are involved in chromatin silencing, Orc1 removal from chromatin, and regulation of chromatin organization [Ageing Signatures]. Ageing-upregulated genes are associated with chromatin binding [Figure 13 H. sapiens Ageing].

DR-essential genes are commonly associated with deacetylase activity, histone deacetylation, and histone H3 deacetylation [Figure 32 Network of Associations Common to DR-Essential Gene Across Species]. However the DR state is associated with global hyperacetylation [Anti-Ageing Drugs]. Acetylation of H3 at lysine 9, 27, 56 is 20-30% higher in dietary restriction animals (Kawakami, et al., 2012). A cluster of deacetylase inhibitors was found to target DR genes [DR Genes Targeting Drugs]. Others sites or only a specific target site subset might be more deacetylated, for instance sirtuins act on lysine 16 of histone H4 (Fraga & Esteller, 2007).

Genes differentially expressed upon DR are commonly involved in chromatin and chromatin organization. Genes upregulated upon DR are involved commonly in covalent chromatin modification. Genes downregulated upon DR are commonly involved in nuclear chromatin, chromatin binding, promoter-specific chromatin binding, chromatin modifying enzymes, chromatin remodelling, and ATP-dependent chromatin remodelling [Dietary Restriction Signatures; Figure 40 R. norvegicus Dietary Restriction]. DR-upregulated genes are associated with nucleosome [Figure 43 C. elegans Dietary Restriction] and chromatin silencing [Figure 39 H. sapiens Dietary Restriction].

Therefore, it appears that chromatin suppression counteracts ageing and ageing downregulates chromatin silencing, while DR upregulates chromatin silencing.

7.4.2 DNA Methylation

Ageing-suppressor genes are commonly involved in DNA methylation, DNA methylation involved in embryo development, and maintenance of DNA methylation. Gerontogenes are commonly involved in regulation of DNA methylation [Ageing Genes].

Genes upregulated during ageing are involved in maintenance of DNA methylation [Ageing Signatures; Figure 17 M. musculus Ageing]. Genes upregulated during cellular senescence are involved in DNA methylation as well as DNA methylation or demethylation. Genes downregulated upon DR are commonly involved in DNA methylation as well as DNA methylation or demethylation [Dietary Restriction Signatures; Figure 39 H. sapiens Dietary Restriction].

Ageing-suppressor genes are involved in DNA hypermethylation or more specifically in hypermethylation of CpG island [Ageing Genes]. Genes upregulated during ageing as well as upon DR are involved in hemi-methylated and double-stranded methylated DNA binding [Ageing Signatures; Dietary Restriction Signatures; Figure 40 R. norvegicus Dietary Restriction].

Ageing is associated with global hypomethylation and loci specific hypermethylation (Johnson, et al., 2012). DR counteracts the age-related hypomethylation (Kim, et al., 2016; Ions, et al., 2013). Both gerontogenes and ageing-suppressor genes act on DNA methylation. Gerontogenes tend to be acting like regulators of DNA methylation.

7.4.3 Histone Modifications

Ageing genes are involved in histone binding and regulation of histone modification [Ageing Genes]. Ageing-suppressor genes, gerontogenes and genes upregulated with ageing or upon DR are all also associated with histone modification [Ageing Genes; Ageing Signatures; Dietary Restriction Signatures].

Histone H4K16 acetylation, H4K20 trimethylation and H3K4 trimethylation all increase with age, while H3K9 methylation and H3K2 trimethylation decrease (Fraga & Esteller, 2007; Han & Brunet, 2012). Chromatin remodelling complexes such as NuRD decrease with ageing (Pegoraro, et al., 2009; Pollina & Brunet, 2011) and other like HP1alpha increase with ageing (Happel, et al., 2008).

7.4.3.1 Histone Acetylation

Ageing genes are involved in regulation of histone acetylation, negative regulation of histone acetylation, histone deacetylase complex, and NAD-dependent histone deacetylase activity. Ageing-suppressor genes suppress histone acetylation and enhance histone deacetylation. Ageing-suppressor genes are associated with histone deacetylation and histone deacetylase activity [Ageing Genes].

Genes downregulated with ageing and upon DR exhibit histone acetyltransferase binding [Figure 13 H. sapiens Ageing; Dietary Restriction Signatures]. DR-essential genes are associated with histone deacetylation, histone deacetylase activity, histone H3 deacetylation, histone deacetylase activity H3-K9 specific, NAD-dependent histone deacetylase activity, and NAD-dependent histone deacetylase activity H3-K9 specific [Dietary Restriction Genes]. DR downregulates histone acetyltransferase binding [Figure 39 H. sapiens Dietary Restriction; Figure 40 R. norvegicus Dietary Restriction], but upregulates histone deacetylase binding, histone H3 deacetylation and histone methyltransferase binding [Dietary Restriction Signatures]. DR-upregulated genes are associated with histone deacetylase binding, but also in particular with histone H3 deacetylation [Figure 40 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction] and more specific with positive regulation of histone deacetylation [Figure 41 M. musculus Dietary Restriction]. Moreover, DR-upregulated genes are associated with histone H3 and H4 acetylation [Figure 41 M. musculus Dietary Restriction].

Trichostatin A is a potent and specific histone deacetylase inhibitor that was found to mimic the gene expression of changes induced by dietary restriction while also reversing the gene expression changes associated with ageing and Hutchinson–Gilford progeria syndrome (HGPS) [Figure 45 Ageing Reversing

Drugs; Figure 52 HGPS Reversing Drugs; Figure 54 DR Mimetics]. Trichostatin A increases the lifespan of model organisms such as *Drosophila* and leads to the induction of heat shock proteins (Tao, et al., 2004).

The acetylation and deacetylation is majorly governed by ageing-suppressor genes.

7.4.3.2 Histone Methylation

Ageing genes are located in histone methyltransferase complex, are involved in histone H3-K4 methylation as well as exhibit histone-lysine N-methyltransferase activity and histone methyltransferase activity H3-K4 specific. Gerontogenes are associated with histone methylation (histone methyltransferase activity, histone-lysine N-methyltransferase activity, and histone methyltransferase activity H3-K9 specific) [Ageing Genes].

Genes downregulated with ageing are involved in demethylation, macromolecule methylation, protein demethylation, histone demethylation, histone lysine demethylation, and histone H3-K27 demethylation as well as histone demethylase activity, demethylase activity, and histone demethylase activity H3-K27 specific. Genes differentially expressed and upregulated upon DR exhibit histone methyltransferase binding [Ageing Signatures]. DR-upregulated genes are associated with methyltransferase activity [Figure 40 R. norvegicus Dietary Restriction] and histone methyltransferase binding [Figure 41 M. musculus Dietary Restriction].

Histone methylation/demethylation seems mostly governed by gerontogenes. Ageing downregulates demethylation, while DR upregulates methyltransferases.

7.4.3.3 Histone Phosphorylation

Ageing-suppressor genes are commonly associated with positive regulation of histone phosphorylation, while gerontogenes are involved in histone phosphorylation itself [Ageing Genes].

Ageing differential expresses (both up and down) histone phosphorylation. Genes differentially expressed with ageing (up- and downregulated genes together) exhibit histone kinase activity and are involved in regulation of histone phosphorylation as well as positive regulation of histone phosphorylation. Genes downregulated during ageing are associated with histone-threonine phosphorylation, histone H3-T6 phosphorylation and exhibit histone kinase activity H3-T6 specific. Cellular senescence differentially expresses histone phosphorylation (in particular histone-serine phosphorylation), downregulates histone kinase activity, and upregulates histone H2A phosphorylation, regulation of histone phosphorylation and positive regulation of histone phosphorylation [Ageing Signatures. DR-essential genes exhibit histone kinase activity [Dietary Restriction Genes].

Therefore, histone phosphorylation appears ambiguous regarding its affects on ageing/lifespan. Certain histone phosphorylation seem to be gerontogenic (and associated with cellular senescence such as H2A phosphorylation), while others may by ageing-suppressive (e.g. H3-T6) depend on the type of histone and residues that are modified.

7.4.3.4 Chromatin Remodelling

Ageing genes including gerontogenes, ageing suppressor genes and also DR-essential genes are all involved in chromatin remodelling [Ageing Genes]. Genes upregulated with ageing are involved in chromatin remodelling too [Ageing Signatures]. Genes downregulated with DR are involved in chromatin remodelling and specifically ATP-dependent chromatin remodelling as well as chromatin remodelling at centromere [Dietary Restriction Signatures]. Further, DR-essential genes are also involved in chromatin remodelling [Dietary Restriction Genes].

The NuRD complex that is a histone deacetylase complex associated is with both gerontogenes and ageing-suppressor genes as well as ageing-upregulated genes [Figure 17 M. musculus Ageing] and DR-essential genes. Piccolo NuA4 histone acetyltransferase complex is associated with gerontogenes [Ageing Genes] and DR-downregulated genes are associated with NuA4 histone acetyltransferase complex too [Figure 41 M. musculus Dietary Restriction]. MLL5-L complex is also associated with genes downregulated upon DR [Figure 41 M. musculus Dietary Restriction].

7.5 Proteostasis Loss

Proteostasis (i.e. protein homeostasis) involves synthesis, folding, processing, trafficking, aggregation, disaggregation and degradation of proteins (Powers, et al., 2009). Loss of protein homeostasis during ageing may lead to impaired protein solubility and cellular dysfunction (Reis-Rodrigues, et al., 2012). All cells exhibit a number of quality control mechanisms to preserve the stability of their proteome.

Post-translational protein modification is associated with ageing-downregulated genes and *de novo* post-translational protein folding is associated with ageing-upregulated genes [Ageing Signatures].

7.5.1 Translation

Protein biosynthesis occurs via translation at ribosomes. Ageing genes are commonly associated with the ribosome. Ribosomal protein S6 kinase activity in particular is associated with gerontogenes. Translation is commonly associated with gerontogenes as well. More specifically gerontogenes are associated with participating in translational initiation, tRNA aminoacylation for protein translation, formation of translation preinitiation complex, and regulation of translational initiation, exhibiting translation initiation factor activity and being located in the eukaryotic translation initiation factor 3 complex [Ageing Genes; Figure 7 C. elegans Ageing Gene Network].

It seems important to note that gerontogenes do not only strongly associate with ribosome binding and translation, but with the regulation of translation and in particular positive regulation of translation. Gerontogenes are associated with all phases of positive regulation of translation from initiation through elongation to termination, including exhibiting translation elongation factor activity [Ageing Genes]. Gerontogenes are also associated with translational frame-shifting that is a mechanism whereby different proteins may result from a single mRNA molecule, due to a change in the parsing of three nucleotides per codon relative to an initiating AUG codon [Ageing Genes]. To the contrary, ageing-suppressor genes are associated with the negative regulation of ribosome biogenesis as well as circadian regulation of translation [Ageing Genes].

Genes downregulated with ageing are associated with translation and regulation of translation, while genes differentially expressed with ageing are involved in positive regulation of translation [Ageing Signatures]. Structural constituent of ribosomes and translation are associated with ageing-differentially expressed and downregulated genes [Figure 17 M. musculus Ageing] as well as is tRNA aminoacylation for protein translation [Figure 20 S. cerevisiae Ageing]. Ageing downregulated genes are also associated with selenocysteine incorporation [Figure 13 H. sapiens Ageing]. These associations with downregulation could be just be part of the general decline of function.

Genes upregulated upon DR are associated with regulation of translation and genes differentially expressed with ageing are associated with circadian regulation of translation (similar to ageing-suppressor genes). However, DR-downregulated genes are associated with cytosolic small ribosomal subunit [Figure 39 H. sapiens Dietary Restriction], and therefore DR could suppress translation by limiting a ribosomal subunit. DR essential genes are associated with being involved in translation, regulation of translation, positive regulation of translation, negative regulation of translational initiation and even regulation of mitochondrial translation, and being located in the eukaryotic translation initiation factor 4F complex [Dietary Restriction Genes].

Therefore overall ageing does not seem to work by transcriptionally regulating the translational machinery, but rather translation appears to be a modulator (like dietary restriction utilizes) to affect something that mediates ageing.

Ageing genes and ageing-suppressor genes in particular are associated with being involved in post-translational protein modification [Figure 5 M. musculus Ageing Gene Network].

7.5.2 Chaperoning

Molecular chaperones are a class of proteins that assist the covalent folding/unfolding and assembly/disassembly of other micromolecular structures. Most chaperones, but not all, are heat-shock proteins that are expressed in response to elevated temperatures or cellular stresses.

Ageing genes are commonly associated with chaperone-mediated protein folding requiring a cofactor [Figure 10 Common Ageing Gene Network]. Ageing-suppressor genes are associated with iron chaperone activity and positive regulation of chaperone-mediated protein complex assembly. Ageing-suppressor genes are also associated with the endoplasmic reticulum unfolded protein response [Ageing Genes; Figure 19 C. elegans Ageing] .

Ageing-differentially expressed genes are associated with chaperone binding [Figure 13 H. sapiens Ageing]. Protein binding involved in protein folding and chaperone mediated protein folding requiring a cofactor are commonly associated with ageing-differentially expressed genes. Protein folding is commonly associated with ageing-downregulated genes [Figure 10 Common Ageing Gene Network]. Ageing-upregulated genes have been found to be significantly associated with exhibiting chaperone binding [Figure 13 H. sapiens Ageing]. Common to ageing-upregulated genes there is a significant association of being involved in the cellular response to heat [Figure 18 D. melongaster Ageing; Figure 21 Common Ageing].

DR-upregulated genes are associated with protein binding involved in protein folding, and chaperone-mediated protein folding [Figure 39 H. sapiens Dietary Restriction]. DR-downregulated genes exhibit chaperone mediated protein folding requiring cofactor [Dietary Restriction Signatures].

Ageing-suppressor genes are commonly involved in protein repair [Ageing Genes]. DR-essential genes are involved protein repair as well [Dietary Restriction Genes]. Protein repair is the process of restoring a protein to its original state after damage by for instance oxidation or spontaneous decomposition of residues.

7.5.3 Aggregates

If proteins are misfolded they tend to form various kinds of aggregates. These aggregates accumulate and disrupt cellular function.

The aggresome is associated with ageing genes and aggresome assembly is associated with gerontogenes [Ageing Genes]. Genes upregulated with ageing are commonly significantly associated with being located in the aggresome [Ageing Signatures; Figure 13 H. sapiens Ageing]. DR-essential genes and DR-downregulated gene are both significantly associated with aggresome and aggresome assembly.

Ageing-suppressor genes significantly interact with APP [Ageing Genes]. Further, ageing-differentially expressed genes (up and down) [Ageing Signatures; Figure 13 H. sapiens Ageing; Figure 13 M. musculus Ageing] and DR-downregulated genes (but not upregulated genes) [Dietary Restriction Signatures] interact significantly with APP.

Gerontogenes promote aggresome assembly, while DR downregulates the assembly of aggresomes. The association of amyloid beta with ageing-suppressor genes might indicate that ageing-suppressors prevent the deleterious affect of amyloid beta or that amyloid beta suppresses the activity of ageing-suppressor genes, as it is the case for telomerase. The downregulation of genes interacting with APP upon DR could be due to a less need to handle amyloid beta aggregates.

7.5.4 Proteolytic Systems

There are two primary proteolytic systems in cells that destroy damaged or misfolded proteins. These are the ubiquitin-proteasome system and the lysosomal autophagy. Both the ubiquitin-proteasome system and autophagy decline with ageing (Rubinsztein, et al., 2011; Tomaru, et al., 2012).

7.5.5 Ubiquitin Proteasome System

Ageing genes are associated with negative regulation of proteolysis. Ageing-suppressor genes are associated with being involved in regulation of protein ubiquitination [Figure 6 D. melanogaster Ageing Gene Network] and ubiquitin-dependent protein catabolic processes [Figure 7 C. elegans Ageing Gene Network; Figure 10 Common Ageing Gene Network]. Ageing-suppressor genes are significantly associated with Cul4A-RING E3 ubiquitin ligase complex. Proteasome-mediated ubiquitin-dependent protein catabolic process is associated with ageing-suppressor genes [Ageing Genes].

Proteasome binding, and being located in the proteasome complex are both associated with ageing-downregulated genes [Figure 17 M. musculus Ageing]. Ageing-upregulated genes are significantly participating in negative regulation of endopeptidase activity [Figure 13 H. sapiens Ageing]. Protein polyubiquitination is associated with ageing-differentially expressed genes. Ubiquitin protein ligase binding is associated with ageing-downregulated genes [Ageing Signatures].

The ubiquitin-proteasome system is associated with ageing-downregulated genes with regulation of proteasomal protein catabolic process, and protein deubiquitination, and exhibiting proteasome binding, as well as proteasome complex [Ageing Signatures; Figure 17 M. musculus Ageing]. Positive regulation of protein ubiquitination is associated with DR-essential genes [Dietary Restriction Genes] and the ubiquitin protein ligase binding was found to be associated with DR-upregulated genes [Dietary Restriction Signatures].

7.5.6 Ubiquitin

Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly involved in proteasome-mediated ubiquitin-dependent protein catabolic process and positive regulation of proteasomal ubiquitin-dependent protein catabolic process. Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved in protein ubiquitination and exhibit ubiquitin protein ligase binding as well as ubiquitin-like protein ligase binding. Gerontogenes do not have any unique associations with ubiquitin-related processes, function, or locations. Ageing-suppressor genes however are commonly involved in ubiquitin-dependent protein catabolic process, protein ubiquitination involved in ubiquitin-dependent protein catabolic process, thiol-dependent ubiquitin-specific protease activity, ubiquitin ligase complex, ubiquitin-protein transferase activity, positive regulation of ubiquitin-protein transferase activity, ubiquitin-like protein transferase activity, ubiquitin-like protein-specific protease activity, ubiquitin mediated proteolysis, ubiquitin mediated degradation of phosphorylated Cdc25A, autodegradation of the E3 ubiquitin ligase COP1, ubiquitinyl hydrolase activity, thiol-dependent ubiquitinyl hydrolase activity, deubiquitination, and protein deubiquitination [Ageing Genes]. DR-essential genes commonly exhibit ubiquitin protein ligase binding and ubiquitin-like protein ligase binding. Protein ubiquitination involved in ubiquitin-dependent protein catabolic process, ubiquitin conjugating enzyme binding, ubiquitin conjugating enzyme activity, ubiquitin-like protein conjugating enzyme binding, ubiquitin-like protein conjugating enzyme activity, ubiquitin protein ligase binding, ubiquitin-like protein ligase binding, positive regulation of ubiquitin-protein transferase activity, ubiquitin-specific protease binding, ubiquitin mediated proteolysis, positive regulation of protein ubiquitination, positive regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process, deubiquitination, and protein ubiquitination are all associated with DR-essential genes [Dietary Restriction Genes].

Ageing-differentially expressed genes (up- and down-regulated genes together) are commonly involved in regulation of protein ubiquitination, protein polyubiquitination, ubiquitin mediated proteolysis, and positive regulation of proteasomal ubiquitin-dependent protein catabolic process. Ageing differentially expressed genes (both up- and down-regulated genes) are commonly involved in ubiquitin protein ligase binding and ubiquitin-like protein ligase binding. Genes upregulated with ageing are involved in protein ubiquitination and positive regulation of protein ubiquitination. Genes downregulated with ageing are involved in thiol-dependent ubiquitin-specific protease activity, ubiquitin-like protein-specific protease activity, ubiquitin-dependent protein catabolic process, ubiquitin-dependent degradation of cyclin D and cyclin D1, ubiquitinyl hydrolase activity, thiol-dependent ubiquitinyl hydrolase activity, deubiquitination, and protein deubiquitination. Genes upregulated in HGPS are involved in protein K48-linked ubiquitination. Cellular senescence up- and down-regulated genes are both commonly involved ubiquitin binding and ubiquitin-like protein binding. Genes upregulated in cellular senescence are involved in ubiquitin protein ligase binding, ubiquitin-like protein ligase binding, ubiquitin-like protein-specific protease activity, thiol-dependent ubiquitin-specific protease activity, thiol-dependent ubiquitinyl hydrolase activity, regulation of ER-associated ubiquitin-dependent protein catabolic process, negative regulation of ER-associated ubiquitin-dependent protein catabolic process, ubiquitinyl hydrolase activity, deubiquitination, protein K11-linked deubiquitination, protein K63-linked deubiquitination, and protein K48-linked deubiquitination. Genes downregulated in cellular senescence are involved in protein ubiquitination, negative regulation of protein ubiquitination, regulation of protein ubiquitination, positive regulation of protein ubiquitination, ubiquitin-dependent protein catabolic process, proteasome-mediated

ubiquitin-dependent protein catabolic process, regulation of proteasomal ubiquitin-dependent protein catabolic process, positive regulation of proteasomal ubiquitin-dependent protein catabolic process, negative regulation of proteasomal ubiquitin-dependent protein catabolic process, protein ubiquitination involved in ubiquitin-dependent protein catabolic process, regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process, positive regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process, regulation of ubiquitin protein ligase activity, positive regulation of ubiquitin protein ligase activity, negative regulation of ubiquitin protein ligase activity, regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle, positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition, negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle, positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition, nuclear ubiquitin ligase complex, regulation of ubiquitin-protein transferase activity, positive regulation of ubiquitin-protein transferase activity, negative regulation of ubiquitin-protein transferase activity, and protein deubiquitination [Ageing Signatures].

Genes differentially expressed upon DR (up- and down-regulated genes together) are commonly involved in protein ubiquitination, ubiquitin-dependent protein catabolic process. There are no ubiquitin-related processes, functions, or locations that are associated commonly to DR-upregulated and DR-downregulated genes. Genes downregulated upon DR commonly exhibit ubiquitin protein ligase binding and ubiquitin-like protein ligase binding. Genes upregulated upon DR are commonly exhibit ubiquitin binding and ubiquitin-like protein binding, as well as are involved in regulation of protein ubiquitination, negative regulation of protein ubiquitination, and deubiquitination [Dietary Restriction Signatures].

Ubiquitin-related processes, functions and locations seem to be specific for ageing-suppressor genes. Protein ubiquitination could be upregulated during ageing because of the greater amount of misfolded and modified proteins, that have to be tagged for degradation, but are overloading the protease/lysosomal systems. In DR conditions, in contrast, the levels of misfolded and adversarial modified proteins is much lower and create therefore less need to ubiquitinate proteins. Overall, ageing does downregulate more ubiquitin-related processes, functions and locations than it upregulates, while DR does upregulate more than it downregulates. Similar to ageing, cellular senescence downregulates many more ubiquitin-related processes, functions and locations than it upregulates. All this indicates that ubiquitination is generally a protective and ageing-suppressing process. Some ubiquitinations are very specific for signalling though and could have ageing promoting effects.

7.5.7 Proteolysis

Ageing genes are commonly involved in proteolysis. Ageing-suppressor genes are commonly involved in ubiquitin mediated proteolysis. Gerontogenes are involved in negative regulation of proteolysis [Ageing Genes].

Genes differentially expressed with ageing are commonly involved in proteolysis, and negative as well as positive regulation of proteolysis. Genes upregulated with ageing are involved in regulation of proteolysis, regulation of membrane protein ectodomain proteolysis and negative regulation of proteolysis. Genes downregulated with ageing are involved in cellular protein catabolic process and regulation of proteolysis involved in cellular protein catabolic process [Ageing Signatures]. Genes upregulated with DR are also involved in proteolysis [Dietary Restriction Signatures].

7.5.8 Proteases

Ageing genes are commonly involved in Ub-specific processing proteases, ubiquitin-like protein-specific protease activity, and thiol-dependent ubiquitin-specific protease activity. Protease binding and Ub-specific processing proteases are associated with gerontogenes. Ub-specific processing proteases, ubiquitin-like protein-specific protease activity, and thiol-dependent ubiquitin-specific protease activity, protease binding, ubiquitin-specific protease binding, and SUMO-specific protease activity are all associated with ageing-suppressor genes [Ageing Genes]. DR-essential genes are significantly associated with Ub-specific processing proteases and ubiquitin-specific protease binding [Dietary Restriction Genes].

Genes differentially expressed with ageing are involved in Ub-specific processing proteases. Genes downregulated with ageing commonly exhibit protease binding, ubiquitin-like protein-specific protease activity, thiol-dependent ubiquitin-specific protease activity [Ageing Signatures]. Genes upregulated in HGPS exhibit protease binding. Genes upregulated upon DR are involved in Ub-specific processing proteases [Dietary Restriction Signatures].

HGPS differentially expressed genes (both up and downregulated genes) are involved in proteolysis, regulation of proteolysis and positive regulation of proteolysis. Genes downregulated in HGPS are involved in negative regulation of proteolysis [Ageing Signatures].

7.5.9 Proteasome

Ageing genes are commonly involved in proteasome-mediated ubiquitin-dependent protein catabolic process, exhibit proteasome-activating ATPase activity, and are located in proteasome regulatory particle base subcomplex as well as nuclear proteasome complex. Ageing-suppressor genes are commonly associated with proteasome complex, proteasome accessory complex, proteasome regulatory particle, proteasome 19S regulatory particle PA700, and proteasome regulatory particle base subcomplex [Ageing Genes].

Genes downregulated with ageing commonly exhibit ageing proteasome binding and are located in the proteasome complex [Ageing Signatures]. Proteasome, proteasome-mediated ubiquitin-dependent protein catabolic process, antigen processing - ubiquitination and proteasome degradation, GLI3 is processed to GLI3R by the proteasome, and degradation of GLI1 by the proteasome are all downregulated during ageing. Genes downregulated in cellular senescence are involved in proteasome-mediated ubiquitin-dependent protein catabolic process [Ageing Signatures].

DR-essential genes are involved in degradation of GLI1 by the proteasome [Dietary Restriction Genes]. Genes upregulated upon DR are involved in proteasome-mediated ubiquitin-dependent protein catabolic process [Dietary Restriction Signatures].

7.5.10 Autophagy

Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved in animal autophagy, CVT pathway, regulation of autophagy, and positive regulation of macroautophagy. Gerontogenes are commonly associated with negative regulation of autophagy and macroautophagy. Ageing-suppressor genes are commonly associated with autophagy, regulation of autophagy, mitophagy, Pink1/Parkin mediated mitophagy, and, macroautophagy, and nucleophagy (including piecemeal microautophagy of nucleus and late nucleophagy) [Ageing Genes].

Ageing genes commonly are associated with regulation of autophagy, where ageing-suppressor genes are associated with autophagy including macroautophagy and mitophagy as well as positive regulation of autophagy. Gerontogenes on the other hand negatively regulate autophagy [Figure 10 Common Ageing Gene Network].

Ageing-differentially expressed genes (up- and down-regulated genes together) are commonly involved in animal autophagy and regulation of chaperone-mediated autophagy. Genes upregulated with ageing are involved in autophagy and negative regulation of autophagy. Genes differentially expressed in HGPS are involved in regulation of autophagy. Genes differentially expressed in cellular senescence (both up- and down-regulated genes) are involved in regulation of autophagy. Genes downregulated in cellular senescence are involved in regulation of autophagy, positive regulation of autophagy, regulation of macroautophagy, and positive regulation of macroautophagy [Ageing Signatures].

DR-essential genes are commonly involved in autophagy, animal autophagy, CVT pathway, macroautophagy, positive regulation of macroautophagy, piecemeal microautophagy of nucleus, and late nucleophagy [Figure 32 Network of Associations Common to DR-Essential Gene Across Species]. DR-essential genes are also involved in yeast autophagy, regulation of autophagy, positive and negative regulation of autophagy, mitophagy, regulation of macroautophagy, glycophagy, pexophagy, and reticulophagy [Dietary Restriction Genes].

Autophagy is associated with DR-upregulated genes and negative regulation of macroautophagy is associated with DR-downregulated genes [Dietary Restriction Signatures]. Genes differentially expressed upon DR (both up- and down-regulated genes) are commonly involved in animal autophagy. Late endosomal microautophagy is associated with DR-differentially expressed genes (up- and down-regulated genes together). Chaperone-mediated autophagy, protein targeting to vacuole involved in autophagy, and protein targeting to lysosome involved in chaperone-mediated autophagy are associated with DR-downregulated genes. Regulation of chaperone-mediated autophagy is associated with DR-upregulated genes [Dietary Restriction Signatures].

DR modulates autophagy. The downregulation of chaperone-mediated autophagy and protein targeting to lysosome as well as overexpression of regulation of chaperone-mediated autophagy upon DR can be attributed to a reduced need of misfolded or modified proteins. DR-essential genes are strongly associated with various forms of autophagy and notably both forms of nucleophagy.

Rapamycin suppresses translation while it induced autophagy via inhibition of TOR. Rapamycin targets both ageing and DR-essential genes, reverses ageing, mimics DR, reverses HGPS and cellular senescence. Overexpression of LAMP2, encoding the receptor for chaperone-mediated autophagy, prevents age-related decline in autophagic activity and improves hepatic function with ageing (Zhang & Cuervo, 2008). Spermidine selectively induces macroautophagy by inhibiting certain histone acetylases and extends the lifespan in yeast, worm, fly, mouse and human cells in culture (Eisenberg, et al., 2009; Eisenberg, et al., 2016). Resveratrol can also trigger autophagy by activating histone deacetylases and extends the lifespan of nematode worms (Morselli, et al., 2009). The positive effects of either spermidine or resveratrol are lost when essential autophagic modulators are inactivated. Protein hypoacetylation seems to be necessary for lifespan extension with these interventions.

Proteostasis is altered with ageing (Koga, et al., 2011). Chronic expression of unfolded, misfolded or aggregated proteins contribute to age-related pathologies, such as Alzheimer's disease, Parkinson's disease and cataracts (Powers, et al., 2009).

7.6 Nutrient Sensing Deregulation

Growth (both organismal and cellular) need to be coordinated according to nutrient availability. Metabolism is regulated at multiple levels by hormone actions, via feedback and control mechanisms. The somatotropic axis consists of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II) as well as their associated carrier proteins and receptors. Other hormones such as insulin, leptine, glucocorticoids and thyroid hormones are involved in modulating GH and/or IGF-I synthesis and availability (Renaville, et al., 2002). IGF-I uses the same receptors as insulin. Therefore, it is common to refer to this pathway collectively as Insulin/IGF-I signalling (IIS).

7.6.1 Growth Hormone

Gerontogenes are involved in somatotropin secreting cell development and differentiation. Growth hormone receptor binding, signalling, and signalling pathway are commonly associated with ageing-suppressor genes. However, gerontogenes are involved in growth hormone secretion, growth hormone receptor binding, and positive regulation of growth hormone secretion. Gerontogenes are also involved in growth hormone-releasing hormone activity, growth hormone-releasing hormone receptor binding, and growth hormone-releasing hormone receptor activity [Ageing Genes].

DR-essential genes are also involved in somatotropin secreting cell differentiation. DR-essential genes are involved in growth hormone receptor complex, growth hormone receptor activity, growth hormone receptor signalling, growth hormone receptor signalling pathway, and negative regulation of growth hormone secretion [Dietary Restriction Genes].

Genes differentially expressed with ageing (up- and down-regulated genes together) are commonly involved in growth hormone receptor signalling, response to growth hormone, and cellular response to growth hormone stimulus [Ageing Signatures].

Genes upregulated upon DR are involved in growth hormone receptor signalling, while positive regulation of growth hormone secretion and response to growth hormone are associated with DR-differentially expressed genes and accelerated ageing differentially expressed genes, respectively [Dietary Restriction Signatures].

7.6.2 Insulin/IGF Signalling

Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly associated with regulation of insulin receptor signalling pathway and positive regulation of insulin-like growth factor receptor signalling pathway. Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved in signalling by insulin receptor, insulin receptor signalling pathway, insulin receptor signalling cascade, insulin receptor recycling, positive and negative regulation of insulin receptor signalling pathway, insulin-like growth factor receptor binding, insulin-like growth factor receptor signalling pathway, and signalling by type 1 insulin-like growth factor 1 receptor IGF1R. Ageing-suppressor genes are commonly involved in insulin signalling pathway, signalling by type 1 insulin-like growth factor 1 receptor IGF1R, and negative regulation of insulin secretion involved in cellular response to glucose stimulus. Gerontogenes are commonly involved in regulation of insulin secretion insulin binding, insulin receptor binding, insulin receptor complex, insulin-activated receptor activity, insulin receptor substrate binding, and cellular response to insulin stimulus [Ageing Genes].

Genes differentially expressed during ageing (up- and down-regulated together) exhibit regulation of insulin secretion involved in cellular response to glucose stimulus, insulin binding, insulin-like growth factor receptor binding, negative regulation of insulin receptor signalling pathway, negative regulation of cellular response to insulin stimulus, and positive regulation of insulin secretion involved in cellular response to glucose stimulus. Ageing-differentially expressed genes (up- and down-regulated together) are involved in regulation of insulin secretion and insulin resistance. Genes downregulated during ageing exhibit insulin receptor binding, response to insulin, and cellular response to insulin stimulus. Genes upregulated during ageing are associated with insulin-like growth factor binding, regulation of cellular response to insulin stimulus, and regulation of insulin receptor signalling pathway [Ageing Signatures].

DR-essential genes are associated with regulation of insulin secretion, glucagon-like peptide-1 GLP1 regulates insulin secretion, insulin receptor signalling pathway, insulin receptor signalling cascade, insulin-activated receptor activity, signalling by insulin receptor, insulin-like growth factor receptor signalling pathway, and signalling by type 1 insulin-like growth factor 1 receptor IGF1R [Dietary Restriction Genes]. Unsurprisingly dietary restriction genes are associated with insulin receptor signalling pathway and insulin receptor binding [Figure 29 D. melanogaster DR-Essential Gene Network].

Genes differentially expressed upon DR (up- and down-regulated genes together) exhibit insulin-like growth factor II binding and are involved in regulation of insulin secretion and response to insulin. DR-differentially expressed genes (both up- and down-regulated genes) are involved in regulation of insulin-like growth factor IGF transport and uptake by insulin-like growth factor binding proteins IGFBPs, insulin signalling pathway, insulin resistance, regulation of insulin receptor signalling pathway, negative regulation of insulin receptor signalling pathway, and negative regulation of cellular response to insulin stimulus. DR-upregulated genes exhibit insulin receptor binding, insulin-like growth factor binding and insulin-like growth factor I binding [Dietary Restriction Signatures].

IGF1 is associated with downregulated genes during development and associated with differentially expressed genes in HGPS. IGF1R is associated with upregulated genes during ageing. IGF2 receptor (IGF2R) and binding proteins (IGF2B1/2/3) are associated with upregulated genes during development, but associated with downregulated genes in HGPS. IGF2BP1 is associated with downregulation during ageing [Ageing Signatures]. IGF2 is associated with genes downregulated upon DR [Dietary Restriction Signatures].

Gerontogenes are associated with somatotropin secreting cell development and differentiation [Figure 5 M. musculus Ageing Gene Network] as well as the insulin receptor signalling pathway [Figure 7 C. elegans Ageing Gene Network], insulin-activated receptor activity, insulin-like growth factor receptor binding and insulin stimulus [Figure 10 Common Ageing Gene Network].

Ageing-differentially expressed genes are associated with insulin receptor binding, the cellular response to insulin stimulus, insulin-like growth factor receptor signalling pathway, and positive regulation of insulin secretion. Ageing-upregulated genes are associated with cellular response to insulin stimulus, and insulin-like growth factor I binding. Ageing-downregulated genes are associated with negative regulation of insulin receptor signalling pathway [Ageing Signatures; Figure 13 H. sapiens Ageing].

GH and IGF1 levels decline during normal ageing as well as in a premature ageing model (Schumacher, et al., 2008). Suppression of IIS at the transcript level during ageing could be a compensation for the declining hormone levels, but at the same time promoting ageing with all its negative downstream side effects.

7.6.3 TOR

Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved mTOR signalling pathway and energy dependent regulation of mTOR by LKB1-AMPK. Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly involved in TORC1 signalling and positive regulation of TOR signalling. Gerontogenes are commonly involved in TOR signalling, mTOR signalling, and mTORC1-mediated signalling as well as located in TOR, TORC1, and TORC2 complex. Ageing-suppressor genes are commonly involved in negative regulation of TOR and TORC1 signalling [Ageing Genes].

Genes upregulated during development are involved in regulation of TOR signalling. Genes differentially expressed during ageing (both up- and down-regulated genes) are involved in regulation of TOR signalling. Genes downregulated during ageing are involved in mTOR signalling pathway and negative regulation of TOR signalling [Ageing Signatures].

DR-essential genes are commonly involved in TOR signalling, mTOR signalling, mTOR signalling pathway, energy dependent regulation of mTOR by LKB1-AMPK, negative regulation of TOR signalling, as well as TOR and TORC1 complex [Dietary Restriction Genes]. Genes differentially expressed upon DR (up- and down-regulated genes together) are involved in regulation of TOR signalling. DR-differentially expressed genes (both up- and down-regulated genes) are involved in TOR signalling and participate in PI3K/Akt/mTOR signalling pathway. Genes upregulated upon DR are involved in mTOR signalling pathway. Genes downregulated upon DR interact with MTOR, RICTOR, and RPTOR, are involved in TORC1 signalling, mTOR signalling, and mTORC1-mediated signalling, as well as TORC1 and TORC2 complex [Dietary Restriction Signatures].

From this it is clear that TOR signalling is gerontogenic, regulated already during development, upregulated during ageing and downregulated upon DR.

7.6.4 AMPK

Ageing genes (both gerontogenes and ageing-suppressor genes) are involved in AMPK signalling pathway. Ageing-suppressor genes commonly participate in AMPK inhibits ChREBP transcriptional activation activity [Ageing Genes]. Genes downregulated during ageing are commonly involved in AMPK signalling pathway [Ageing Signatures]. DR-essential genes are commonly participating in energy dependent regulation of mTOR by LKB1-AMPK. AMPK signalling pathway and AMPK inhibits ChREBP transcriptional activation activity are associated with DR-essential genes [Dietary Restriction Genes]. Genes upregulated upon DR are commonly involved in AMPK signalling pathway [Dietary Restriction Signatures].

The adenosine monophosphate-activated protein kinase (AMPK) function as a nutrient sensor that mediates signalling from nutrient scarcity (such as created under DR conditions) and catabolism to retard ageing systems. AMPK is therefore like a sensor of cellular energy levels. A high cellular ratio of AMP/ATP triggers the phosphorylation and hence activation of AMPK. Activated AMPK in turn phosphorylates a wide array of target proteins including ChREBP (Carbohydrate Response Element Binding Protein), which inactivation by phosphorylation reduces transcription of key enzymes of glycolytic and lipogenic pathways (Cheung, et al., 2000; Kawaguchi, et al., 2002; Hardie, et al., 2003; Hardie, 2004). AMPK activation may mediate the lifespan-extending and health-promoting effect of metformin which was found here to target

specifically gerontogenes, to reverse ageing gene expression changes and to mimic the gene expression signature of DR.

7.6.5 Sirtuins

Genes downregulated with ageing are interacting with SIRT1 [Ageing Signatures]. SIRT2, SIRT4, and SIRT5 and are all downregulated during ageing [Ageing Signatures]. Genes upregulated in cellular senescence are participating in SIRT1 negatively regulates rRNA expression [Ageing Signatures]. DR-essential genes commonly participate in SIRT1 negatively regulates rRNA expression [Dietary Restriction Genes]. Genes downregulated upon DR are participating in SIRT1 negatively regulates rRNA expression [Dietary Restriction Signatures].

Sirtuins are also nutrient sensors that sense the NAD/NADH ratio. Sirtuins are a class of enzymes that possess either mono-ADP-ribosyltransferase or NAD-dependent deacetylase activity (class III) that among other targets deacetylates histones and epigenetically silence gene expression. When glucose is low, NAD is high (compared to low NADH), then the activity of SIRT1 is high which in turn reduces the activity of its target genes such as ribosomal genes.

7.6.6 FOXOs

FoxO signalling pathway is commonly associated with both gerontogenes and ageing-suppressor genes. AKT-mediated inactivation of FOXO1A is commonly associated with gerontogenes [Ageing Genes]. Genes differentially expressed (both up- and down-regulates genes) during ageing or cellular senescence are participating in FoxO signalling pathway. Genes downregulated in cellular senescence interact with FOXO1 and FOXO3 [Ageing Signatures]. DR-essential genes participate in FoxO signalling pathway and AKT-mediated inactivation of FOXO1A [Dietary Restriction Genes]. Genes upregulated upon DR are commonly participating in FoxO signalling pathway [Dietary Restriction Signatures].

FOXO signalling pathway contains both positive and negative regulators of FOXO transcription factors. FOXO3 is a DR-essential genes. FOXO1A also mediates the anticancer effect of DR.

7.7 Signalling Alteration

7.7.1 MAPK

Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly involved in activation of MAPK activity and ERK/MAPK targets. Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved in MAPK signalling pathway, MAPK family signalling cascades, negative regulation of MAPK pathway, gastrin-CREB signalling pathway via PKC and MAPK, and FCERI mediated MAPK activation. Gerontogenes are commonly involved in MAPK signalling pathway and MAPK1/MAPK3 signalling. Ageing-suppressor genes are commonly involved in MAPK cascade, MAPK signalling pathway, activation of MAPKK activity, MAPK6/MAPK4 signalling, MAPK JNK signalling, MAPK ERK1/2 signalling, MAPK1 ERK2 activation, MAP2K and MAPK activation, RAF-independent MAPK1/3 activation, regulation of stress-activated MAPK cascade, MAPK targets nuclear events mediated by MAP kinases, and negative feedback regulation of MAPK pathway. Therefore, ageing-suppressor genes are strongly associated with MAP kinase activity [Ageing Genes; Figure 10 Common Ageing Gene Network].

Ageing downregulated genes are also associated with MAPK cascade [Figure 21 Common Ageing]. Genes differentially expressed during ageing (both up- and down-regulated genes together) are commonly involved in MAPK signalling pathway, MAPK family signalling cascades, and positive regulation of MAPK cascade. Genes differentially expressed during ageing (both up- and down-regulated genes) are commonly involved in Inactivation of MAPK activity, activation of MAPKK activity, and negative regulation of MAPK cascade. Genes upregulated during ageing are commonly involved in regulation of stress-activated MAPK cascade, regulation of MAPK cascade, activation of MAPK activity, positive regulation of stress-activated MAPK cascade, and positive regulation of ERK1 and ERK2 cascade. Genes downregulated during ageing are commonly involved in Gastrin-CREB signalling pathway via PKC and MAPK. Genes differentially expressed during ageing (both up- and down-regulated genes) are commonly involved in MAPK signalling pathway, and regulation of MAPK cascade [Ageing Signatures].

DR-essential genes are commonly involved in activation of MAPK activity, MAPK6/MAPK4 signalling [Dietary Restriction Genes]. Genes downregulated upon DR are associated with MAPK cascade, activation of MAPK activity, positive regulation of MAPK cascade, and positive regulation of ERK1 and ERK2 cascade [Dietary Restriction Signatures; Figure 45 Common Dietary Restriction].

MAPK signalling seems to have more associations with ageing-suppressor genes, but ageing-suppressor are also involved in negative regulation of MAPK signalling. Therefore MAPK appears to have both gerontogenic as well as ageing-suppressing functions. However MAPK signalling is enhanced during ageing and suppressed upon DR. This may indicate that its gerontogenic activities outcompete its ageing-suppressing activities. It was suggested that the main pathway used by spermidine to trigger its effects is the MAPK pathway (Minois, 2014). Both ageing as well as DR appear to modulate MAPK signalling (Zhen, et al., 1999).

7.7.2 Wnt

Ageing genes (both gerontogenes and ageing-suppressor genes) are commonly involved in Wnt signalling pathway. Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly involved in signalling by Wnt. Ageing-suppressor genes are commonly involved in TCF dependent signalling in response to WNT [Ageing Genes].

Genes differentially expressed during ageing (up- and down-regulated genes together) are involved in repression of WNT target genes and Wnt signalling pathway calcium modulating pathway. Genes differentially expressed during ageing (both up- and down-regulated genes) are involved in regulation of Wnt signalling pathway, regulation of canonical Wnt signalling pathway, negative regulation of Wnt signalling pathway, positive regulation of Wnt signalling pathway, and positive regulation of canonical Wnt signalling pathway. Genes upregulated during ageing are involved in regulation of non-canonical Wnt signalling pathway, negative regulation of non-canonical Wnt signalling pathway, and regulation of Wnt signalling pathway planar cell polarity pathway. Genes downregulated during ageing are commonly involved in Wnt signalling pathway, regulation of Wnt signalling pathway, regulation of canonical Wnt signalling pathway, negative regulation of Wnt signalling pathway, negative regulation of canonical Wnt signalling pathway, beta-catenin independent WNT signalling, cell-cell signalling by Wnt, and WNT5A-dependent internalization of FZD4. Genes downregulated during ageing are interacting with WNT1, exhibit Wnt-protein binding, and are involved in signalling by Wnt, Wnt signalling pathway, Wnt signalling network, canonical Wnt signalling pathway, non-canonical Wnt signalling pathway, negative regulation of canonical Wnt signalling pathway, cell-cell signalling by Wnt, presenilin action in Notch and Wnt signalling, TCF dependent signalling in response to WNT, regulation of Wnt-mediated beta catenin signalling and target gene transcription, beta-catenin independent WNT signalling, WNT5A-dependent internalization of FZD4, negative regulation of TCF-dependent signalling by WNT ligand antagonists, Wnt signalling pathway planar cell polarity pathway, canonical Wnt signalling pathway involved in positive regulation of apoptotic process, and canonical Wnt signalling pathway involved in negative regulation of apoptotic process [Ageing Signatures].

DR-essential genes are involved in Wnt signalling pathway and canonical Wnt signalling pathway [Dietary Restriction Genes]. Genes downregulated upon DR are commonly involved in regulation of canonical Wnt signalling pathway, regulation of Wnt signalling pathway, negative regulation of canonical Wnt signalling pathway, and negative regulation of Wnt signalling pathway [Dietary Restriction Signatures].

Certain aspects of Wnt signalling appear to be gerontogenic, while others are ageing-suppressing. Overall, Wnt signalling is greatly suppressed during ageing. DR clearly desuppresses Wnt signalling. Wnt signalling may also be DR-essential.

7.7.3 Notch

Gerontogenes are commonly associated with Notch signalling pathway. Gerontogenes interact with NOTCH1 and are participating in Notch-mediated HES/HEY network and positive regulation of Notch signalling pathway. Ageing-suppressor genes are involved in Notch receptor processing, presenilin action in Notch and Wnt signalling and negative regulation of Notch signalling pathway [Ageing Genes].

Genes downregulated during ageing are involved in presenilin action in Notch and Wnt signalling, regulation of Notch signalling pathway, and negative regulation of Notch signalling pathway. Genes downregulated in HGPS are interacting with NOTCH3. Genes upregulated in HGPS are interacting with NOTCH1 and NOTCH2, exhibit Notch binding, and are involved in Notch receptor processing, Notch signalling pathway, regulation of Notch signalling pathway, positive regulation of Notch signalling pathway, signalling by NOTCH, signalling by NOTCH1, signalling by NOTCH1 in cancer, signalling by NOTCH1 PEST domain mutants in cancer, signalling by NOTCH1 HDPEST domain mutants in cancer, signalling by NOTCH1 HD domain mutants in cancer, constitutive signalling by NOTCH1 HDPEST domain mutants, constitutive signalling by NOTCH1 PEST domain mutants, signalling by NOTCH1 t(7;9)(NOTCH1:M1580 K2555) translocation mutant, constitutive signalling by NOTCH1 HD domain mutants, constitutive signalling by NOTCH1 t(7;9)(NOTCH1:M1580 K2555) translocation mutant, signalling by NOTCH2, signalling by NOTCH3, signalling by NOTCH4, Notch signalling involved in heart development, activated NOTCH1 transmits signal to the nucleus, and NOTCH2 activation and transmission of signal to the nucleus [Ageing Signatures].

Genes upregulated upon DR are commonly involved in Notch signalling pathway and negative regulation of Notch signalling pathway. DR-upregulated genes are involved in regulation of Notch signalling pathway. DR-downregulated genes are involved in Notch signalling pathway, presenilin action in Notch and Wnt signalling, signalling by NOTCH1, and activated NOTCH1 transmits signal to the nucleus [Dietary Restriction Signatures].

Notch signalling is gerontogenic. HGPS extensively positively regulates Notch signalling. However, normal ageing also activates Notch signalling, while DR shuts down Notch.

7.8 Mitochondrial Dysfunction

Mitochondria are crucial organelles for energy production via oxidative phosphorylation. During ageing, the efficacy of the respiratory transport chain diminishes, resulting in increased electron leakage, enhanced ROS generation and reduced ATP synthesis (Green, et al., 2011). With increasing age some mitochondria become dysfunctional.

7.8.1 Reactive Oxygen Species

Progressive mitochondrial dysfunction can result in an increased production of free radicals and reactive oxygen species (Harman, 1965).

Ageing genes (gerontogenes and ageing-suppressor genes together) are involved in superoxide radicals degradation and age-dependent response to reactive oxygen species. Gerontogenes are commonly involved in reactive oxygen species metabolic process and response to reactive oxygen species [Ageing Genes]. Ageing-suppressor genes are commonly associated with removal of superoxide radicals [Figure 10 Common Ageing Gene Network] as well as oxidoreductase activity acting on superoxide radicals as acceptor [Ageing Genes]. Ageing-suppressor genes are commonly involved in regulation of reactive oxygen species metabolic process, negative regulation of reactive oxygen species biosynthetic process, and detoxification of reactive oxygen species [Ageing Genes]. Ageing-suppressor genes are associated with negative regulation of reactive oxygen species, metabolic processes and removal of free radicals [Ageing Genes; Figure 5: M. musculus Ageing Gene Network].

Genes differentially expressed with ageing (up- and down-regulated genes together) are involved in reactive nitrogen species metabolic process, reactive oxygen species biosynthetic process, negative regulation of reactive oxygen species metabolic process, reactive oxygen species metabolic process, negative regulation of reactive oxygen species biosynthetic process, cellular response to reactive nitrogen species, and detoxification of reactive oxygen species. Genes differentially expressed with ageing (both up- and down-regulated genes) are involved in response to reactive oxygen species, cellular response to reactive oxygen species, regulation of reactive oxygen species metabolic process, and regulation of reactive oxygen species biosynthetic process [Ageing Signatures]. Ageing-downregulated genes are significantly associated with being involved in regulation of reactive oxygen species, biosynthetic processes and generation of precursor metabolites and energy [Figure 16 R. norvegicus Ageing] as well as exhibiting oxidoreductase activity acting on the CH-OH group of donors with NAD or NADP as the

acceptor [Ageing Signatures; Figure 20 S. cerevisiae Ageing]. Genes upregulated with ageing are commonly involved in regulation of reactive oxygen species biosynthetic process, positive regulation of reactive oxygen species biosynthetic process, and positive regulation of reactive oxygen species metabolic process [Ageing Signatures]. HGPS downregulated genes are involved in removal of superoxide radicals, response to oxygen radical, and cellular response to oxygen radical [Ageing Signatures].

DR-essential genes are commonly associated with the response to oxidative stress [Figure 29 D. melanogaster DR-Essential Gene Network; Figure 32 Network of Associations Common to DR-Essential Gene Across Species] and regulation of reactive oxygen species metabolic process [Dietary Restriction Genes]. Genes differentially expressed upon DR (up- and downregulated genes) are commonly involved in reactive oxygen species metabolic process. Genes downregulated upon DR are commonly involved in response to reactive oxygen species, regulation of reactive oxygen species metabolic process, regulation of reactive oxygen species biosynthetic process, positive regulation of reactive oxygen species biosynthetic process, and positive regulation of reactive oxygen species metabolic process [Dietary Restriction Signatures].

Overall, gerontogenes do enhance free radical generation, while ageing-suppressor suppress radical generation and enhance free radical removal. Ageing promotes generation of free radical, but DR suppresses the generation of free radicals. Accelerated ageing suppresses the combating of free radicals.

7.8.2 Mitochondrial Integrity & Biogenesis

Gerontogenes are commonly associated with the mitochondrion and in particular the mitochondrial inner membrane. Ageing-suppressor genes are also involved in mitochondrion organization and regulation of the mitochondrial membrane potential [Ageing Genes; Figure 10 Common Ageing Gene Network].

Ageing downregulated genes are associated with being located in the mitochondrion and the mitochondrial envelope, but also with the tricarboxylic acid cycle, mitochondrial translation and the mitochondrial large ribosomal subunit [Ageing Signatures]. To the contrary, DR-upregulated genes are associated with being located in the mitochondrion. DR-downregulated genes are significantly associated with participating in positive regulation of protein targeting to the mitochondrion [Dietary Restriction Signatures].

Ageing-suppressor genes are commonly associated with mitophagy, the autophagic process in which mitochondria are transported to the vacuole/lysosome and degraded in response to changing cellular conditions [Ageing Genes]. Mitochondrial fission is associated with ageing-suppressor genes and DR-upregulated genes [Ageing Genes; Dietary Restriction Signatures], while regulation of mitochondrial fission is associated with gerontogenes [Ageing Genes].

Ageing genes (gerontogenes and ageing-suppressor genes together) are involved in transcriptional activation of mitochondrial biogenesis [Ageing Genes]. DR-essential genes are involved in mitochondrial biogenesis and transcriptional activation of mitochondrial biogenesis [Dietary Restriction Genes].

Ageing-suppressor genes are involved in mitochondrial fission and positive regulation of mitochondrial fission. Gerontogenes are involved in regulation of mitochondrial fission and negative regulation of mitochondrial fission. In contrast, gerontogenes are associated with mitochondrial fusion and ageing-suppressor genes in negative regulation of mitochondrial fusion [Ageing Genes].

Genes upregulated during development are involved in regulation of mitochondrial fission and positive regulation of mitochondrial fission. Genes downregulated during ageing are commonly involved in regulation of mitochondrial fission. Positive regulation of mitochondrial fission is associated with ageing-downregulated genes. Similar genes downregulated in cellular senescence are involved in regulation of mitochondrial fission and positive regulation of mitochondrial fission [Ageing Signatures]. Genes upregulated with DR are involved in mitochondrial fission [Dietary Restriction Signatures].

Therefore, it appears that mitochondrial biogenesis is associated with ageing genes and may be DR-essential. Promotion of fission may antagonize ageing, while fusion accelerates it. Although development positively regulates fission, ageing and cellular senescence negative regulate fission of mitochondria. DR counteracts ageing and promotes mitochondrial fission.

7.9 Cellular Senescence

Ageing genes (gerontogenes and ageing-suppressor genes together) are associated with senescence-associated secretory phenotype (SASP). Ageing genes (both gerontogenes and ageing-suppressor genes are involved in cellular senescence. Ageing-suppressor genes are associated with negative regulation of cellular senescence, replicative senescence, oxidative stress induced senescence and DNA damage/telomere stress induced senescence. Gerontogenes are involved in animal organ senescence, replicative senescence, oxidative stress-induced premature senescence, oncogene induced senescence, DNA damage/telomere stress induced senescence, regulation of cellular senescence, positive/negative regulation of cellular senescence, formation of senescence-associated heterochromatin foci (SAHF) senescence-associated secretory phenotype [Ageing Genes].

Genes differentially expressed with ageing are commonly involved in senescence and cellular senescence. Senescence-associated heterochromatin focus is also associated with ageing-differentially expressed gene. Genes upregulated with ageing are involved in oncogene induced senescence [Ageing Signatures].

Genes differentially expressed in cellular senescence (both up- and down-regulated genes) are involved in senescence, cellular senescence, DNA damage/telomere stress induced senescence, and senescence-associated secretory phenotype. Genes upregulated in cellular senescence are involved in oxidative stress induced senescence, oncogene-induced cell senescence, regulation of cellular senescence, positive regulation of cellular senescence, formation of senescence-associated heterochromatin foci, senescence-associated heterochromatin focus, and senescence-associated heterochromatin focus assembly [Ageing Signatures].

DR-essential genes are involved in animal organ senescence, cellular senescence, stress-induced premature senescence, oxidative stress induced senescence, senescence-associated secretory phenotype, negative and positive regulation of cellular senescence [Dietary Restriction Genes]. Genes downregulated upon DR are involved in senescence, cellular senescence, oxidative stress induced senescence, DNA damage/telomere stress induced senescence, and senescence-associated secretory phenotype. Further regulation of phagocytosis is commonly associated with DR-downregulated genes, while phagocytosis recognition and engulfment is commonly associated with DR-upregulated genes [Dietary Restriction Signatures].

Ageing genes are surprisingly strongly involved in cellular senescence where ageing-suppressors are negative regulators and gerontogenes are positive regulators of senescence and various facets/aspects of cellular senescence. Clearly cellular senescence is a gerontogenic process as also evident from experiments that genetically or pharmacological elimination of senescence cells in rodent model organisms increases health and lifespan. Unsurprisingly, but also reinsuring, cellular senescence itself greatly upregulates various aspects of cellular senescence.

Surprisingly again and similar to ageing genes, DR-essential genes are associated with cellular senescence. Confirming this association on the genetic level, the gene activity changes upon DR indicate that DR does not only modulate aspects of cellular senescence but also clearly downregulates cellular senescence. Note that DR genes can be proven by both gain- and loss of function experiments that cancel out the lifespan extending effect of DR. Therefore, it is expected to have both positive and negative regulators, i.e. modulators of the ageing processes via the DR mechanisms. More interestingly DR genes are associated with stress-induced premature senescence. So while ageing genes also modulate the genetic mechanisms of replicative senescence, DR genes modulate cellular senescence and in particular in the response to stress.

The number of senescence cells increases with age, although it might not be true for all types of tissues (Wang, et al., 2009b). Dietary restriction is effective in delaying cellular senescence (de Cabo, et al., 2015) and reducing the number of senescence cells (Wang, et al., 2010). The accumulation of senescent cells with ageing can reflect an increased rate of generation of senescence cells an/or a decreased rate of clearance that could be the consequence of an attenuated immune response and/or overwhelmed immune system. This could mean that DR does maybe not only reduce the rate of generation of cellular senescence but it could also increase the rate that senescence cells are actively removed. Indeed there is

evidence that senescence cells, just like senescence tumour cells, are under strict immune surveillance and efficiently removed by phagocytosis (Hoenicke & Zender, 2012; Kang, et al., 2011b; Xue, et al., 2007).

The p16(INK4a)/Rb and p19(ARF)/p53 are the most established pathways among the different mechanisms that implement cellular senescence. p16(INK4a) and p19(ARF) are encoded by the same gene CDKN2A/Cdkn2a. CDKN2A/Cdkn2a is commonly upregulated in mammalian consensus signatures of ageing. The levels of p16(INK4a) and, to a lesser extent also p19(ARF), correlate with the chronological age of all essential tissues analysed in both human (Ressler, et al., 2006) and mice (Krishnamurthy, et al., 2004). Further, ageing genes (both gerontogenes and ageing-suppressor genes) interact significantly specifically with CDKN2A and CDKN2A is also associated with DR-downregulated genes.

7.10 Stem Cell Exhaustion

Ageing is accompanied with decline in the regenerative potential of tissues that results in the most obvious phenotypes such as greying of the hair. Several biological immortal organisms exhibit unlimited regenerative abilities.

Ageing genes (gerontogenes and ageing-suppressor genes together) are commonly involved in germ-line stem cell division. Ageing-suppressor genes are commonly involved in positive regulation of stem cell proliferation. Hematopoietic stem cell proliferation and negative regulation of hematopoietic stem cell differentiation are associated with ageing-suppressor genes as well. Neurogenesis is associated with gerontogenes, while positive regulation of neurogenesis is associated with ageing-suppressor genes. Germ-line stem-cell niche homeostasis is associated with gerontogenes [Ageing Genes].

Ageing-differentially expressed genes are associated with stem cell development and male germ-line stem cell asymmetric division [Ageing Signatures]. Genes that are differentially expressed with ageing (both up- and down-regulated with ageing) are commonly involved in stem cell differentiation and neurogenesis. Genes differentially expressed with ageing (up- and down-regulated with ageing together) are commonly involved in regulation of stem cell proliferation, including positive regulation of stem cell proliferation and regulation of neurogenesis. Genes upregulated with ageing are commonly involved in negative regulation of neurogenesis [Ageing Signatures; Figure 16 R. norvegicus Ageing]. Hematopoietic progenitor cell differentiation and positive regulation of hematopoietic stem cell proliferation, differentiation and migration are associated with ageing-upregulated genes. Ageing-upregulated genes are associated with hematopoietic progenitor cell differentiation and positive regulation of hematopoietic stem cell migration [Ageing Signatures].

Genes differentially expressed in HGPS are involved in stem cell population maintenance, stem cell proliferation, stem cell differentiation, regulation of stem cell differentiation, positive regulation of stem cell differentiation, and signalling events mediated by stem cell factor receptor c-Kit. Genes upregulated in HGPS are involved in stem cell development, stem cell division, somatic stem cell division, neuronal stem cell population maintenance, and negative regulation of stem cell differentiation. Genes downregulated in HGPS are involved in stem cell fate commitment, somatic stem cell population maintenance, hematopoietic stem cell differentiation, regulation of stem cell proliferation, hematopoietic stem cell proliferation, positive/negative regulation of stem cell proliferation, signalling pathways regulating pluripotency of stem cells, and transcriptional regulation of pluripotent stem cells [Ageing Signatures].

DR-essential genes are involved in male germ-line stem cell asymmetric division and neuronal stem cell population maintenance [Dietary Restriction Genes]. Genes differentially expressed upon DR (up- and down-regulated genes together) are commonly involved in regulation of stem cell proliferation including positive regulation of stem cell proliferation. DR-upregulated genes are commonly associated with regulation of neurogenesis. DR-upregulated genes are associated with negative regulation of hematopoietic stem cell differentiation [Dietary Restriction Signatures].

Stem cell related processes and functions are mostly associated with ageing-suppressors genes in particular positive regulation of stem cell maintenance. Ageing does downregulate stem cell maintenance. Accelerated ageing in particular greatly modulates stem cell processes and functions including downregulation of stem cell maintenance and stemness. Notable, DR modulates stem cell regulation and may therefore enhance stem cell functions. From the data alone it is clear that stem cells are counteracting ageing. Somatic stem cell maintenance including neurogenesis diminishes significantly with increasing

age (Gray, et al., 2002). Hematopoiesis declines with age that results in a diminished production of adaptive immune cells, leading to immunosenescence and increased likelihood of developing in anemia and myeloid malignancies (Shaw, et al., 2010).

7.11 Intercellular Communication Alteration

Ageing in multicellular organisms involves also changes in the communication between cells and systemic factors that include endocrine, paracrine neuroendocrine, and neuronal factors. The most notable is that of the phenomenon designated as inflammageing that is a slowly developing proinflammatory phenotype that accompanies ageing in mammals (Salminen, et al., 2012).

Ageing genes (gerontogenes and ageing-suppressor genes together) are involved in regulation of inflammatory response and positive regulation of inflammatory response. Gerontogenes are involved in inflammatory response, inflammatory mediator regulation of TRP channels, positive regulation of acute inflammatory response, and positive regulation of leukotriene production involved in inflammatory response. Ageing-suppressor genes are involved in acute inflammatory response, negative regulation of inflammatory response, regulation of cytokine production involved in inflammatory response, negative regulation of respiratory burst involved in inflammatory response, and DExH-box helicases activate type I IFN and inflammatory cytokines production. Further I-kappaB kinase activity and complex are associated with gerontogenes. Ageing-suppressor genes are associated with negative regulation of cytokine production, regulation of cytokine secretion involved in immune response, regulation of cytokine production involved in inflammatory response and macrophage cytokine production. Gerontogenes are associated with the cellular response to cytokine stimulus, positive regulation of cytokine secretion/production and myeloid leukocyte cytokine production involved in immune response, cytokine activity and receptor binding [Ageing Genes].

Genes differentially expressed during ageing (up- and down-regulated genes together) are commonly involved in positive regulation of acute inflammatory response, regulation of inflammatory response to antigenic stimulus, positive regulation of inflammatory response to antigenic stimulus, positive regulation of acute inflammatory response to antigenic stimulus, inflammatory mediator regulation of trp channels, leukocyte migration involved in inflammatory response, and chronic inflammatory response. Genes differentially expressed during ageing (both up- and down-regulated genes) are commonly involved in inflammatory response, and regulation of acute inflammatory response. Genes downregulated during ageing are commonly involved in negative regulation of inflammatory response. Genes upregulated during ageing are commonly involved in acute inflammatory response, regulation of inflammatory response, and positive regulation of inflammatory response, as well as participate in inflammatory bowel disease. Ageing-upregulated genes are associated with cytokine activity [Ageing Signatures].

Genes differentially expressed in cellular senescence (both up- and down-regulated) are involved in inflammatory response and regulation of inflammatory response. Genes differentially expressed in cellular senescence (up- and down-regulated genes together) are involved in the IPAF inflammasome. Genes upregulated in cellular senescence are involved in acute inflammatory response, regulation of acute inflammatory response, positive regulation of inflammatory response, positive regulation of acute inflammatory response, inflammatory response to antigenic stimulus, acute inflammatory response to antigenic stimulus, inflammatory cell apoptotic process, inflammatory response to wounding, wound healing involved in inflammatory response, connective tissue replacement involved in inflammatory response wound healing, CLEC7A inflammasome pathway, inflammatory mediator regulation of TRP channels, and inflammatory bowel disease [Ageing Signatures].

DR genes are associated with macrophage cytokine production and cytokine receptor activity [Dietary Restriction Genes]. Genes differentially expressed upon DR are commonly involved in regulation of cytokine production involved in inflammatory response. Genes downregulated upon DR are involved in acute inflammatory response, regulation of inflammatory response, regulation of acute inflammatory response, and positive regulation of inflammatory response, as well as participates in inflammatory bowel disease. DR-upregulated genes are associated with the regulation of cytokine production and regulation of cytokine production involved in the inflammatory response. DR-downregulated genes are associated with a positive regulation of the inflammatory response, positive regulation of macrophage cytokine production,

positive regulation of cytokine secretion and cytokine activity. Chronic inflammatory response and chronic inflammatory response to antigenic stimulus are downregulated upon DR [Dietary Restriction Signatures].

From this, it is clear that inflammation (especially chronic inflammation) is gerontogenic, i.e. promoted by gerontogenes and counteracted by ageing-suppressors. Ageing as well as cellular senescence promote inflammation, whereas DR downregulates inflammation.

7.12 Rhythm Alteration

Organisms have biological clocks. Some clocks such as the telomeres and epigenetic DNA methylation clock are linear but entrained by cycles. Telomeres are shortened with each cell cycle, while the epigenetic DNA methylation clock is entrained by something else unknown as it is also observed (i.e. it ticks also) in postmitotic tissues. Biological cycles are rhythms that are molecular, physiological, or behavioural events recurring and are practically an ubiquitous property of all living beings. In fact, rhythmicity coordinates biological systems and synchronizes them with the external environment. Biological rhythms occur on different time scales/orders. A simple division is in three classes: A) Ultradian, B) Circadian, and C) Infradian. Ultradian cycles are of a short duration (within seconds, minutes or hours), while the circadian rhythm has a period of just about a day, and the infradian rhythm are longer than a day, e.g. month or year (i.e. circannual) (Lamont & Amir, 2017).

Hormonal activities as well as metabolism undergo cycles of different scales. The circadian rhythm is the most obvious as it follows day night cycles and determines the sleep wake cycle of many organisms.

Ageing genes are commonly involved in rhythmic behaviour. Ageing genes (both gerontogenes and ageing-suppressors genes) are involved in circadian rhythm, locomotor rhythm and arrhythmogenic right ventricular cardiomyopathy. Gerontogenes are associated with circadian rhythm/behaviour, positive/negative regulation of circadian sleep/wake cycle, circadian sleep/wake cycle non-REM sleep, positive regulation of circadian sleep/wake cycle non-REM sleep and entrainment of the circadian clock by the photoperiod. Ageing-suppressors are commonly involved in circadian rhythm. Ageing-suppressor genes are involved in rhythmic process, rhythmic behaviour, circadian rhythm, regulation of circadian rhythm, positive regulation of circadian rhythm, circadian sleep/wake cycle sleep positive regulation of the circadian sleep/wake cycle, circadian regulation of gene expression, circadian regulation of translation, circadian behaviour, circadian clock, BMAL1/CLOCK NPAS2 activates circadian gene expression, regulation of timing of cell differentiation and rhythmic synaptic transmission [Ageing Genes; Figure 5 M. musculus Ageing Gene Network; Figure 6 D. melanogaster Ageing Gene Network]. Genes downregulated during ageing are commonly involved in circadian rhythm, regulation of circadian rhythm, circadian entrainment, and negative regulation of circadian sleep/wake cycle sleep [Ageing Signatures].

DR-essential genes are associated with rhythmic processes, the circadian rhythm, locomotor rhythm, circadian entrainment, and the circadian regulation of gene expression and negative regulation of circadian sleep/wake cycle sleep [Dietary Restriction Genes]. DR-differentially expressed genes (both up- and down-regulated genes) are commonly involved in circadian regulation of gene expression. DR-differentially expressed genes (up- and down-regulated genes together) are commonly involved circadian regulation of translation. Genes downregulated upon DR are commonly involved in circadian rhythm and circadian clock as well as participate in BMAL1/CLOCK NPAS2 activates circadian gene expression. Genes upregulated upon DR are commonly involved in circadian rhythm, circadian entrainment, regulation of circadian rhythm, and negative regulation of circadian rhythm [Dietary Restriction Signatures; Figure 39 H. sapiens Dietary Restriction; Figure 40 R. norvegicus Dietary Restriction; Figure 41 M. musculus Dietary Restriction; Figure 42 D. melanogaster Dietary Restriction; Figure 45 Common Dietary Restriction].

Both gerontogenes and ageing-suppressor genes are associated with circadian rhythmicity, where the associations are more extensively for ageing-suppressor genes which may indicate that circadian rhythmicity is in general suppressing ageing. This is further supported with the observation that circadian rhythmicity is downregulated with ageing. However, DR does modulate the circadian clock as up- and down-regulated genes have both multiple associations with circadian rhythmicity. It could mean that circadian rhythmicity has also gerontogenic impact that could involve the timing of age-related changes via rhythmic activities that affect the telomere/telomerase system and maybe also the epigenetic clock or

other timings systems. Epigenetic modulation in turn cause changes in transcription and subsequently translation (i.e. gene expression) thereby determining the changes in the phenotype as observed with an ageing organism. This could consequently lead to most of the classes of age-related changes (from molecular, cellular, up to physiological changes). This is not mutually exclusive with the possibility that those other classes of age-related changes are influencing the epigenetic landscape as well. Instances of this could be for example the mechanisms of accelerated ageing and interventions such dietary restriction and pharmaceutical agents that modulate the rate of ageing and lead to a change in the lifespan.

Ageing may simple change gene activities such as gerontogenes become enhanced and ageing-suppressor genes are suppressed. It may also upregulate other genes involved in the same process as gerontogenes and downregulate other genes involved in the processes of ageing-suppressor genes. The utilization of gain- and loss-of-function (as here used with specific subclasses of ageing genes) may be capable of helping to dissect which classes of age-related changes are more causal and which are merely effects. Targeting causal changes for interventions is expected to interfere with the ageing process more effectively (and therefore greatly extend lifespan) than targeting effects that lead to modest lifespan extensions.

Clearly among the many discussed results the main novel results are that there are clear subclasses of ageing genes, namely gerontogenes and ageing-suppressor genes. Those classes have different shared significant associations and are antagonizing each other in common superclasses of their associations (i.e. in positive vs. negative regulation of the same processes/activities). Further a specific subclass of ageing genes is responsible and essential for the effect of dietary restriction to extend lifespan. It was shown here that ageing genes and in particular DR-essential genes are conserved on the molecular level more than expected by chance. Further ageing genes and DR-essential genes are also interacting with each other more than expected purely by chance. The study showed that by utilizing a simple graph theory based method such as guilt-by-association on node degree is effective in predicting novel members of a class of interest (here DR-essential genes) with a high accuracy as validated experimentally within this study. Beyond this the results show that consensus signatures of ageing, HGPS, cellular senescence and dietary restriction, derived with a multivariate approach allows to identify differential expression and significant associations that enable to gather rich insights into possible molecular mechanisms of ageing and its interference. For examples associations that are shared among ageing, HGPS and cellular senescence and how they are contrasted with the effect of dietary restriction as well as what they share in common with the associations with specific ageing classes such as gerontogenes and ageing-suppressors. Lastly this study showed that anti-ageing pharmaceutical agents can be computational predicted by at least two ways. A) by applying the guilt-by-association concept on gene/protein - drug associations as well as B) by matching the here derived consensus signatures with those of small molecules. Thereby it is possible to identify components that target specific class of ageing genes and reverse ageing, HGPS, cellular senescence or mimic the effect of dietary restriction.

8 Conclusions

8.1 Contributions

This thesis classifies ageing genes into gerontogenes and ageing-suppressor genes as well as DR-essential genes (in multiple species). Those gene classes are compared and contrasted based on graph approaches utilizing the guilt-by-association principle and the shortest path. Their common associations across species are identified. New members of discrete classes were predicted and experimentally validated by collaborators. Molecular signatures of ageing, HGPS, cellular senescence as well as dietary restriction and small molecules were derived.

First, this was the first attempt and the most holistic approach to characterize ageing genes and the changes that happen to gene activities during ageing and under DR feeding with an emphasis on identifying small molecule manipulators of ageing as far as known. In this work molecular signatures were derived with a multivariate approach that takes the interactions of genes into account, while most of the ageing-related transcriptional signatures published so far were only based on univariate approaches for determining differential expression. In addition to a superior method of differential expression calculation, the consensus signatures generated here are also based on much greater number of profiles than all previous attempts as it included more than 27 datasets for ageing (de Magalhaes, et al., 2009a) and 21 datasets for dietary restriction (Plank, et al., 2012).

The methodologies described here could be utilized in the future for researching other gene classes related to ageing such as longevity-associated genes or senescence-associated genes or related to other phenomena such as cancer and stemness or stem cell ageing.

Secondly, significant structured data as knowledge was curated in the process of this work which was used in other investigations into the genomics of ageing and its manipulation by diet, e.g. *GenAge*, *GenDR*, *Digital Ageing Atlas*, *LongevityMap* among others (Tacutu, et al., 2013; Wuttke, et al., 2012; Craig, et al., 2015; Budovsky, et al., 2013).

Thirdly, many predictive associations were discovered by the approach adopted here, such as a number of strongly statistical significant associations were put in the context with previous knowledge including known classes of age-related changes. Some results reported here in this thesis have been published (Wuttke & de Magalhaes, 2011; Wuttke, et al., 2012; de Magalhaes, et al., 2012; Fuellen, et al., 2012; Johnson, et al., 2012; Plank, et al., 2012; Wuttke & de Magalhaes, 2012; Debonneuil, et al., 2013; Tacutu, et al., 2013; Fuellen, et al., 2013; Craig, et al., 2015).

Fourthly, this research reviewed ageing, dietary restriction, anti-ageing drugs, and the interrelated disciplines on the intersection of computer science and biology [Computational Approaches; Figure 1 Overlapping Fields]. It also reviewed and documented molecular profiling and signatures of ageing and manipulations of ageing in order to identify anti-ageing interventions.

Lastly, using the results on classes of ageing genes including those essential for DR and the molecular signatures of ageing and dietary restriction, small molecules were predicted that target ageing gene subclasses and/or reverse the molecular signatures associated with ageing, HGPS, and senescence, as well as mimic the molecular signature of dietary restriction. With this concrete interventions to experimental test are established. Based on this molecular background experimental evaluation of these anti-ageing strategies is now feasible.

8.2 Summary

Developing an understanding of the mechanisms that underpin the ageing process has been a persistent effort throughout the recorded history. Ageing is one of the hugest enigma of mankind since a very long time. In the post-genomic era with the rapid increase in biological data and computational capacity, it becomes possible to decipher the ageing process, at least at the molecular level. However its reverse engineering requires a highly systematic and holistic approach that is able to integrate diverse data with relevance to ageing from the molecular level up to that of the whole of physiology, considering eventually also the demographic and population level (Fuellen, et al., 2010; Fuellen, et al., 2012; Fuellen, et al., 2013).

Ageing is a process that is controlled by genes, but is also highly malleable by the environment. Those ageing genes can be classified into gerontogenes and ageing-suppressor genes based on whether their activity promotes or suppresses the ageing process, respectively. Those classes of ageing genes have different strong associations [Ageing Genes]. Gerontogenes are primarily involved in development and growth, the somatropic axis as well as epigenetics (including histone methylation), protein translation, neuron death, and inflammation [Ageing Genes]. Ageing-suppressor genes on the contrary are involved in DNA metabolism (including DNA repair and telomere maintenance), nuclear envelope, chromosome organization, epigenetics (including histone (de)acetylation and chromatin silencing), protein homeostasis, stress response, and cytoskeleton [Ageing Genes].

Even more interesting is that those two classes of genes antagonize the very same associations. Gerontogenes are involved in positive regulation while ageing-suppressor genes are involved in negative regulation of cell proliferation. Gerontogenes are strongly positively associated with translation while ageing suppressor genes are negatively associated with it [Ageing Genes]. Therefore besides having different strong associations, they show clear antagonistic behaviour among their significant associations [Ageing Genes].

Dietary restriction, the most powerful non-genetic intervention that interferes with ageing, is also mediated by specific genes, termed here DR genes, which when manipulated abolish the effect of DR to extend lifespan. DR genes are involved in autophagy, stress response, gene regulation, metabolism, growth and development, apoptosis and epigenetics [Dietary Restriction Genes].

Transcriptomic data from different tissues of different ages can be used to identify robust molecular consensus signatures that reflect observations on ageing. The here established consensus signatures of ageing identified interesting associations that confirm what is known about ageing as well as reveal novel relations [Ageing Signatures].

Ageing-upregulated genes are strongly associated with DNA damage, nuclear envelope organisation, the aggresome, apoptosis as well as inflammation and exosomes. These ageing-upregulated genes reflect reduced genomic instability, epigenetic alterations, loss of proteostasis, mitochondrial dysregulation, and altered intercellular communications with trend to inflammation. Exosomes are a novel aspect not well studied in context of ageing [Ageing Signatures]. Ageing-downregulated genes are linked to DNA binding, metabolism, neurogenesis, hormone response, and transcription as well as the MAPK cascade. This reflects epigenetic alterations, stem cell exhaustion, and altered intercellular communications as well. MAPK signalling seems interesting as it appears to have more frequent associations compared to other well known age-related pathways [Ageing Signatures].

Data on transcriptomic experiments involving dietary restricted individuals and fully fed individuals was presented. This data from various organisms were used to establish consensus signatures representing the effect of DR on the transcriptome [Dietary Restriction Signatures]. DR differentially expressed genes are associated with the stress response, circadian clock, apoptosis and proteolysis systems including autophagy and the ubiquitin-proteasome system. The DR-upregulated genes are primary involved in negative regulation of transcription while the downregulated genes are commonly involved in glucocorticoid response, signalling and transcription factors [Dietary Restriction Signatures].

There are a number of instances in which associations of gerontogenes are shared with ageing-upregulated genes and/or DR-downregulated genes, while associations with ageing-suppressor genes are shared with ageing-downregulated genes and/or DR-upregulated genes. For instance

gerontogenes are associated with inflammation, ageing upregulates inflammation, while DR downregulates inflammation. Those associations on the transcript level closely represent the trends observed at other levels of gene expression as well as at the whole physiology level. These were discussed extensively for each class of age-related change. Another instance is related to autophagy. Ageing-suppressor genes are associated with autophagy, ageing differentially expresses negative regulation of autophagy, while autophagy is associated with DR-upregulated genes and negative regulation of autophagy is associated with gerontogenes and downregulated in DR. It is clear that chronic inflammation is detrimental, but its persistent observation associated with ageing is attenuated under DR conditions, while autophagy is beneficial and attenuated during ageing, but induced by DR feeding.

Gene expression is changing (i.e. genes are changing in their activity level) during ageing, HGPS and senescence as well as in DR feeding. Using ageing genes classes and molecular signatures of ageing, HGPS, and senescence as well as DR, drugs can be identified that target specific ageing genes classes, reverse ageing-related signatures or mimic the response to DR. Within this context a number of small molecules have been identified. Some of these were already known as geroprotectors such as rapamycin, metformin and quercetin, while others are novel but have the potential to ameliorate age-related diseases and/or to modify pathways implicated in lifespan control [Small Molecule Predictions].

8.3 Future Research Directions

A logical extension of this work would be the generation of other molecular signatures [Molecular Profiling to Decipher Ageing]. Other omics data other than transcriptomics such as epigenomics and proteomics as well as metabolomics could be used to generate signatures of other types of biological entities. For epigenomics and proteomics, but only to a certain degree applicable to metabolomics data, it is possible to map the signatures to genes as done here for gene transcripts. For proteomics this is rather straightforward as just like transcripts they are encoded by genes. For epigenetic data such as DNA methylation profiles, these can be mapped to genes that are located in with or closely nearby within a certain genomic distance. However, one would lose information for those differentially methylated locations that are in intergenic regions. A similar problem exist for metabolomics where metabolites can be mapped to genes whose gene products are known to bind these molecules. However, it is not necessary to map all kind of entities to genes.

Another line of research extensions would be the utilization of coexpression (correlation matrices) that can be derived from the gene expression profiles. These can be used to infer causality via network reverse engineering (Opgen-Rhein & Strimmer, 2007). The guilt-by-association as well as the Principal Angle Enrichment Analysis can be extended to the utilization of these coexpression or/and causality relations derived from such profiles [Types of Signatures].

In the research described here, a number of tissue-specific signatures were derived, but combined to consensus signatures for easier interpretation. The tissue-specific signatures could be used to investigate the associations that are common to a certain tissue types, as it is known that ageing utilizes also non-autonomous mechanisms and certain tissue unique effects [Tissue-Specific Signatures].

A class of non-ageing genes can be curated in order to predict ageing genes with classifiers. Hallmarks of ageing co-occur and are interconnected, determining the exact causal network is a challenge. Causal network inference is therefore needed for disentangling causality from effects. As ageing is a phenomenon that is nearly universal and also evolutionary conserved apparently, hallmarks that are not common to all ageing organisms might be less likely related to causality.

Data linking specific ages or age ranges with phenotypes, changes and disease are abundant in the biomedical literature. A knowledge base has been proposed to incorporate such data in a way that it can be searched and reasoned over (Geifman & Rubin, 2012; Geifman & Rubin, 2013). Whole-genome gene expression studies have typically found hundreds to thousands of differential expressed genes (i.e. profiles) during ageing and in response to dietary restriction or other lifespan extending interventions. A key open question is which subset of genes mediate longevity. The emergence of huge volumes of omics data may enable computational approaches to reverse-engineer the process of ageing from data.

9 Bibliography

Abalan, et al., 2010 — Abalan, Francois; Mayo, Willy; Simon, Herve; & Le Moal, Michel (2010). '**Paradoxical effect of severe dietary restriction on Long-Evans rat life span.**' *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung. Journal international de vitaminologie et de nutrition*. 80(6), pp. 386-93

Abdulrehman, et al., 2011 — Abdulrehman, Dario; Monteiro, Pedro Tiago; Teixeira, Miguel Cacho; Mira, Nuno Pereira; Lourenco, Artur Bastos; dos Santos, Sandra Costa; Cabrito, Tania Rodrigues; Francisco, Alexandre Paulo; Madeira, Sara Cordeiro; Aires, Ricardo Santos; Oliveira, Arlindo Limede; Sa-Correia, Isabel; & Freitas, Ana Teresa (2011). '**YEASTRACT: providing a programmatic access to curated transcriptional regulatory associations in Saccharomyces cerevisiae through a web services interface.**' *Nucleic acids research*. 39(Database issue), pp. D136-40.

Aghajan, et al., 2010 — Aghajan, Mariam; Jonai, Nao; Flick, Karin; Fu, Fei; Luo, Manlin; Cai, Xiaolu; Ouni, Ikram; Pierce, Nathan; Tang, Xiaobo; Lomenick, Brett; Damoiseaux, Robert; Hao, Rui; Del Moral, Pierre M; Verma, Rati; Li, Ying; Li, Cheng; Houk, Kendall N; Jung, Michael E; Zheng, Ning; Huang, Lan; Deshaies, Raymond J; Kaiser, Peter; & Huang, Jing (2010). '**Chemical genetics screen for enhancers of rapamycin identifies a specific inhibitor of an SCF family E3 ubiquitin ligase.**' *Nature biotechnology*. 28(7), pp. 738-42.

Ahmed & Tollefsbol, 2003 — Ahmed, Ali & Tollefsbol, Trygve (2003). '**Telomeres, telomerase, and telomerase inhibition: clinical implications for cancer.**' *Journal of the American Geriatrics Society*. 51(1), pp. 116-22.

Akerfelt, et al., 2010 — Akerfelt, Malin; Vihervaara, Anniina; Laiho, Asta; Conter, Annie; Christians, Elisabeth S; Sistonen, Lea; & Henriksson, Eva (2010). '**Heat shock transcription factor 1 localizes to sex chromatin during meiotic repression.**' *The Journal of biological chemistry*. 285(45), pp. 34469-76.

Alavez, et al., 2011 — Alavez, Silvestre; Vantipalli, Maithili C; Zucker, David J S; Klang, Ida M; & Lithgow, Gordon J (2011). '**Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan.**' *Nature*. 472(7342), pp. 226-9.

Alic, et al., 2011 — Alic, Nazif; Andrews, T Daniel; Giannakou, Maria E; Papatheodorou, Irene; Slack, Cathy; Hoddinott, Matthew P; Cocheme, Helena M; Schuster, Eugene F; Thornton, Janet M; & Partridge, Linda (2011). '**Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling.**' *Molecular systems biology*. 7, pp. 502.

Aliper, et al., 2015 — Aliper, Alexander M; Csoka, Antonei Benjamin; Buzdin, Anton; Jetka, Tomasz; Roumiantsev, Sergey; Moskalev, Alexy; & Zhavoronkov, Alex (2015). '**Signaling pathway activation drift during aging: Hutchinson-Gilford Progeria Syndrome fibroblasts are comparable to normal middle-age and old-age cells.**' *Aging*. 7(1), pp. 26-37.

Anand, et al., 2015 — Anand, Deepti; Agrawal, Smriti; Siddam, Archana; Motohashi, Hozumi; Yamamoto, Masayuki; & Lachke, Salil A (2015). '**An integrative approach to analyze microarray datasets for prioritization of genes relevant to lens biology and disease.**' *Genomics data*. 5, pp. 223-227.

Anderson, et al., 2003 — Anderson, Rozalyn M; Bitterman, Kevin J; Wood, Jason G; Medvedik, Oliver; & Sinclair, David A (2003). '**Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae.**' *Nature*. 423(6936), pp. 181-5.

Anderson, et al., 2016 — Anderson, Edward N; Corkins, Mark E; Li, Jia-Cheng; Singh, Komudi; Parsons, Sade; Tucey, Tim M; Sorkac, Altar; Huang, Huiyan; Dimitriadi, Maria; Sinclair, David A; Hart, Anne C (2016). **"C. elegans lifespan extension by osmotic stress requires FUdR, base excision repair, FOXO, and sirtuins."** Mech Ageing Dev. 2016 Mar;154:30-42. doi: 10.1016/j.mad.2016.01.004. Epub 2016 Feb 22.

Anderson & Weindruch, 2012 Anderson, Rozalyn M & Weindruch, Richard (2012). '**The caloric restriction paradigm: implications for healthy human aging.**' *American journal of human biology : the official journal of the Human Biology Council*. 24(2), pp. 101-6.

Anisimov, et al., 2011 Anisimov, Vladimir N; Berstein, Lev M; Popovich, Irina G; Zabezhinski, Mark A; Egormin, Peter A; Piskunova, Tatiana S; Semenchenko, Anna V; Tyndyk, Margarita L; Yurova, Maria N; Kovalenko, Irina G; & Poroshina, Tatiana E (2011). '**If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice.**' *Aging*. 3(2), pp. 148-57.

Anisimov, et al., 2011a Anisimov, Vladimir N; Zabezhinski, Mark A; Popovich, Irina G; Piskunova, Tatiana S; Semenchenko, Anna V; Tyndyk, Margarita L; Yurova, Maria N; Rosenfeld, Svetlana V; & Blagosklonny, Mikhail V (2011). '**Rapamycin increases lifespan and inhibits spontaneous tumorigenesis in inbred female mice.**' *Cell cycle (Georgetown, Tex.)*. 10(24), pp. 4230-6.

Anisimov, et al., 2011b Anisimov, Vladimir N; Egorov, Maxim V; Krasilshchikova, Marina S; Lyamzaev, Konstantin G; Manskikh, Vasily N; Moshkin, Mikhail P; Novikov, Evgeny A; Popovich, Irina G; Rogovin, Konstantin A; Shabalina, Irina G; Shekarova, Olga N; Skulachev, Maxim V; Titova, Tatiana V; Vygodin, Vladimir A; Vyssokikh, Mikhail Yu; Yurova, Maria N; Zabezhinsky, Mark A; & Skulachev, Vladimir P (2011). '**Effects of the mitochondria-targeted antioxidant SkQ1 on lifespan of rodents.**' *Aging*. 3(11), pp. 1110-9.

Anson, et al., 2003 Anson, R Michael; Guo, Zhihong; de Cabo, Rafael; Iyun, Titilola; Rios, Michelle; Hagepanos, Adrienne; Ingram, Donald K; Lane, Mark A; & Mattson, Mark P (2003). '**Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake.**' *Proceedings of the National Academy of Sciences of the United States of America*. 100(10), pp. 6216-20.

Antezana, et al., 2013 Antezana, Erick; Mironov, Vladimir; & Kuiper, Martin (2013). '**The emergence of Semantic Systems Biology.**' *New biotechnology*. 30(3), pp. 286-90.

Antosh, et al., 2011a Antosh, Michael; Fox, David; Helfand, Stephen L; Cooper, Leon N; & Neretti, Nicola (2011). '**New comparative genomics approach reveals a conserved health span signature across species.**' *Aging*. 3(6), pp. 576-83.

Antosh, et al., 2011b Antosh, Michael; Whitaker, Rachel; Kroll, Adam; Hosier, Suzanne; Chang, Chengyi; Bauer, Johannes; Cooper, Leon; Neretti, Nicola; & Helfand, Stephen L (2011). '**Comparative transcriptional pathway bioinformatic analysis of dietary restriction, Sir2, p53 and resveratrol life span extension in Drosophila.**' *Cell cycle (Georgetown, Tex.)*. 10(6), pp. 904-11.

Aouida, et al., 2009 Aouida, Mustapha; Khodami-Pour, Ali; & Ramotar, Dindial (2009). '**Novel role for the Saccharomyces cerevisiae oligopeptide transporter Opt2 in drug detoxification.**' *Biochemistry and cell biology = Biochimie et biologie cellulaire*. 87(4), pp. 653-61.

Aranda, et al., 2010 Aranda, B; Achuthan, P; Alam-Faruque, Y; Armean, I; Bridge, A; Derow, C; Feuermann, M; Ghanbarian, A T; Kerrien, S; Khadake, J; Kerssemakers, J; Leroy, C; Menden, M; Michaut, M; Montecchi-Palazzi, L; Neuhauser, S N; Orchard, S; Perreau, V; Roechert, B; van Eijk, K; & Hermjakob, H (2010). '**The IntAct molecular interaction database in 2010.**' *Nucleic acids research*. 38(Database issue), pp. D525-31.

Arino, et al., 2012 Arino, Toru; Karakawa, Seiji; Ishiwata, Yasuyoshi; Nagata, Masashi; & Yasuhara, Masato (2012). '**Effect of cimetidine on pentamidine induced hyperglycemia in rats.**' *European journal of pharmacology*. 693(1-3), pp. 72-9.

Arking, 2006 Arking, R. (2006) '**The Biology of Aging: Observations and Principles**'. 3rd Ed, Oxford University Press.

Arum, et al., 2009 Arum, Oge; Bonkowski, Michael S; Rocha, Juliana S; & Bartke, Andrzej (2009). '**The growth hormone receptor gene-disrupted mouse fails to respond to an intermittent fasting diet.**' *Aging cell*. 8(6), pp. 756-60.

Ash & Merry, 2011 — Ash, C E & Merry, B J (2011). '**The molecular basis by which dietary restricted feeding reduces mitochondrial reactive oxygen species generation.**' *Mechanisms of ageing and development*. 132(1-2), pp. 43-54.

Ashburner, et al., 2000 — Ashburner, M; Ball, C A; Blake, J A; Botstein, D; Butler, H; Cherry, J M; Davis, A P; Dolinski, K; Dwight, S S; Eppig, J T; Harris, M A; Hill, D P; Issel-Tarver, L; Kasarskis, A; Lewis, S; Matese, J C; Richardson, J E; Ringwald, M; Rubin, G M; Sherlock, G (2000). **"Gene ontology: tool for the unification of biology. The Gene Ontology Consortium."** Nat Genet. 2000 May;25(1):25-9.

Attia, et al., 2015 — Attia, S M; Ahmad, S F; Okash, R M; & Bakheet, S A (2015). '**Dominant lethal effects of nocodazole in germ cells of male mice.**' *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*. 77, pp. 101-4.

Austriaco, 1996 — Austriaco, N R Jr (1996). **"Review: to bud until death: the genetics of ageing in the yeast, Saccharomyces."** Yeast. 1996 Jun 15;12(7):623-30.

Ayyadevara, et al., 2008 — Ayyadevara S, Alla R, Thaden JJ, Shmookler Reis RJ (2008) **Remarkable longevity and stress resistance of nematode PI3K-null mutants.**. *Aging cell*, 7(1), pp.13-22.

Babst, 2011 — Babst, Markus (2011). '**MVB vesicle formation: ESCRT-dependent, ESCRT-independent and everything in between.**' *Current opinion in cell biology*. 23(4), pp. 452-7.

Baker, et al., 1989 — Baker, S J; Fearon, E R; Nigro, J M; Hamilton, S R; Preisinger, A C; Jessup, J M; vanTuinen, P; Ledbetter, D H; Barker, D F; Nakamura, Y; White, R; & Vogelstein, B (1989). '**Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas.**' *Science (New York, N.Y.)*. 244(4901), pp. 217-21.

Baker, et al., 2016 — Baker, Darren J; Childs, Bennett G; Durik, Matej; Wijers, Melinde E; Sieben, Cynthia J; Zhong, Jian; Saltness, Rachel A; Jeganathan, Karthik B; Verzosa, Grace Casaclang; Pezeshki, Abdulmohammad; Khazaie, Khashayarsha; Miller, Jordan D; & van Deursen, Jan M (2016). '**Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan.**' *Nature*. 530(7589), pp. 184-9.

Baker & Sprott, 1988 — Baker, G T 3rd & Sprott, R L (1988). '**Biomarkers of aging.**' *Experimental gerontology*. 23(4-5), pp. 223-39.

Balaji, et al., 2006 — Balaji, S; Babu, M Madan; Iyer, Lakshminarayan M; Luscombe, Nicholas M; & Aravind, L (2006). '**Comprehensive analysis of combinatorial regulation using the transcriptional regulatory network of yeast.**' *Journal of molecular biology*. 360(1), pp. 213-27.

Balderhaar, et al., 2010 — Balderhaar, Henning J Kleine; Arlt, Henning; Ostrowicz, Clemens; Brocker, Cornelia; Sundermann, Frederik; Brandt, Roland; Babst, Markus; & Ungermann, Christian (2010). '**The Rab GTPase Ypt7 is linked to retromer-mediated receptor recycling and fusion at the yeast late endosome.**' *Journal of cell science*. 123(Pt 23), pp. 4085-94.

Barabasi, et al., 2011 — Barabasi, Albert-Laszlo; Gulbahce, Natali; & Loscalzo, Joseph (2011). '**Network medicine: a network-based approach to human disease.**' *Nature reviews. Genetics*. 12(1), pp. 56-68.

Barger, et al., 2008a — Barger, Jamie L; Kayo, Tsuyoshi; Vann, James M; Arias, Edward B; Wang, Jelai; Hacker, Timothy A; Wang, Ying; Raederstorff, Daniel; Morrow, Jason D; Leeuwenburgh, Christiaan; Allison, David B; Saupe, Kurt W; Cartee, Gregory D; Weindruch, Richard; & Prolla, Tomas A (2008). '**A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice.**' *PloS one*. 3(6), pp. e2264.

Barger, et al., 2015 — Barger, Jamie L; Anderson, Rozalyn M; Newton, Michael A; da Silva, Cristina; Vann, James A; Pugh, Thomas D; Someya, Shinichi; Prolla, Tomas A; & Weindruch, Richard (2015). '**A conserved transcriptional signature of delayed aging and reduced disease vulnerability is partially mediated by SIRT3.**' *PloS one*. 10(4), pp. e0120738.

Barger, et al., 2017 — Barger, Jamie L; Vann, James M; Cray, Nicole L; Pugh, Thomas D; Mastaloudis, Angela; Hester, Shelly N; Wood, Steven M; Newton, Michael A; Weindruch, Richard; & Prolla, Tomas A (2017). '**Identification of tissue-specific transcriptional markers of caloric restriction in the mouse and their use to evaluate caloric restriction mimetics.**' *Aging cell*.

Barna, et al., 2000 — Barna, M; Hawe, N; Niswander, L; & Pandolfi, P P (2000). '**Plzf regulates limb and axial skeletal patterning.**' *Nature genetics*. 25(2), pp. 166-72.

Barrows & Kokkonen, 1975 — Barrows, C H Jr & Kokkonen, G (1975). '**Protein synthesis, development, growth and life span.**' *Growth*. 39(4), pp. 525-33.

Bartke, 2008 — Bartke, Andrzej (2008). **"Impact of reduced insulin-like growth factor-1/insulin signaling on aging in mammals: novel findings."** Aging Cell. 2008 Jun;7(3):285-90. doi: 10.1111/j.1474-9726.2008.00387.x. Epub 2008 Mar 11.

Bartke, 2012 — Bartke, Andrzej (2012). **"Healthy aging: is smaller better? - a mini-review."** Gerontology. 2012;58(4):337-43. doi: 10.1159/000335166. Epub 2012 Jan 18.

Barton, et al., 2010 — Barton, C E; Johnson, K N; Mays, D M; Boehnke, K; Shyr, Y; Boukamp, P; & Pietenpol, J A (2010). '**Novel p63 target genes involved in paracrine signaling and keratinocyte differentiation.**' *Cell death & disease*. 1, pp. e74.

Bauer, et al., 2010 — Bauer, Johannes; Antosh, Michael; Chang, Chengyi; Schorl, Christoph; Kolli, Santharam; Neretti, Nicola; & Helfand, Stephen L (2010). '**Comparative transcriptional profiling identifies takeout as a gene that regulates life span.**' *Aging*. 2(5), pp. 298-310.

Bell, et al., 2009 — Bell, Russell; Hubbard, Alan; Chettier, Rakesh; Chen, Di; Miller, John P; Kapahi, Pankaj; Tarnopolsky, Mark; Sahasrabuhde, Sudhir; Melov, Simon; & Hughes, Robert E (2009). '**A human protein interaction network shows conservation of aging processes between human and invertebrate species.**' *PLoS genetics*. 5(3), pp. e1000414.

Benjamini & HochBerg, 1995 — Benjamini, Yoav; Hochberg, Yosef (1995) "**Controlling the false discovery rate: a practical and powerful approach to multiple testing**". *Journal of the Royal Statistical Society*, Series B. 57 (1): 289–300. MR 1325392.

Benyshek, et al., 2006 — Benyshek, D C; Johnston, C S; & Martin, J F (2006). '**Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life.**' *Diabetologia*. 49(5), pp. 1117-9.

Berchtold, et al., 2008 — Berchtold, Nicole C; Cribbs, David H; Coleman, Paul D; Rogers, Joseph; Head, Elizabeth; Kim, Ronald; Beach, Tom; Miller, Carol; Troncoso, Juan; Trojanowski, John Q; Zielke, H Ronald; & Cotman, Carl W (2008). '**Gene expression changes in the course of normal brain aging are sexually dimorphic.**' *Proceedings of the National Academy of Sciences of the United States of America*. 105(40), pp. 15605-10.

Berger, 2007 — Berger, Shelley L (2007). '**The complex language of chromatin regulation during transcription.**' *Nature*. 447(7143), pp. 407-12.

Berger, 2014 — Shelley L. Berger (2014) '**Berger To Discuss Senescence, Aging in Mahoney Lecture**', "Current thinking holds that aging is plastic and its pace can be slowed or even reversed..." Available from <http://nihrecord.nih.gov/newsletters/2014/02_28_2014/story5.htm>

Bernardes de Jesus, et al., 2011 — Bernardes de Jesus, Bruno; Schneeberger, Kerstin; Vera, Elsa; Tejera, Agueda; Harley, Calvin B; & Blasco, Maria A (2011). '**The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence.**' *Aging cell*. 10(4), pp. 604-21.

Bernstein, et al., 2004 — Bernstein, Adam M; Willcox, Bradley J; Tamaki, Hitoshi; Kunishima, Nobuyoshi; Suzuki, Makoto; Willcox, D Craig; Yoo, Ji-Suk Kristen; & Perls, Thomas T (2004). '**First autopsy study of an Okinawan centenarian: absence of many age-related diseases.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 59(11), pp. 1195-9.

Beuming, et al., 2005 — Beuming, Thijs; Skrabanek, Lucy; Niv, Masha Y; Mukherjee, Piali; & Weinstein, Harel (2005). '**PDZBase: a protein-protein interaction database for PDZ-domains.**' *Bioinformatics (Oxford, England)*. 21(6), pp. 827-8.

Bhadra, et al., 2016 Bhadra, Ankan Kumar; Das, Eshita; & Roy, Ipsita (2016). '**Protein aggregation activates erratic stress response in dietary restricted yeast cells.**' *Scientific reports*. 6, pp. 33433.

Bierkamp, et al., 2010 Bierkamp, Christiane; Luxey, Maeva; Metchat, Aicha; Audouard, Christophe; Dumollard, Remi; & Christians, Elisabeth (2010). '**Lack of maternal Heat Shock Factor 1 results in multiple cellular and developmental defects, including mitochondrial damage and altered redox homeostasis, and leads to reduced survival of mammalian oocytes and embryos.**' *Developmental biology*. 339(2), pp. 338-53.

Biocompare, 2013 Biocompare (2013). '**Caloric Restriction Has A Protective Effect On Chromosomes.**'.

Bishop & Guarente, 2007 Bishop, Nicholas A & Guarente, Leonard (2007). '**Genetic links between diet and lifespan: shared mechanisms from yeast to humans.**' *Nature reviews. Genetics*. 8(11), pp. 835-44.

Blasco, 2007 Blasco, Maria A (2007). '**Telomere length, stem cells and aging.**' *Nature chemical biology*. 3(10), pp. 640-9.

Bohnert & Kenyon, 2017 Bohnert KA, Kenyon C. (2017) '**A lysosomal switch triggers proteostasis renewal in the immortal C. elegans germ lineage.**' *Nature*. 2017 Nov 30;551(7682):629-633. doi: 10.1038/nature24620. Epub 2017 Nov 22.

Bois & Grosveld, 2003 Bois, Philippe R J & Grosveld, Gerard C (2003). '**FKHR (FOXO1a) is required for myotube fusion of primary mouse myoblasts.**' *The EMBO journal*. 22(5), pp. 1147-57.

Bonkowski, et al., 2006 Bonkowski, Michael S; Rocha, Juliana S; Masternak, Michal M; Al Regaiey, Khalid A; & Bartke, Andrzej (2006). '**Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction.**' *Proceedings of the National Academy of Sciences of the United States of America*. 103(20), pp. 7901-5.

Bonkowski & Sinclair, 2016 Bonkowski, Michael S & Sinclair, David A (2016). '**Slowing ageing by design: the rise of NAD+ and sirtuin-activating compounds.**' *Nature reviews. Molecular cell biology*. 17(11), pp. 679-690.

Botelho, et al., 2009 Botelho, M C; Crespo, M; Almeida, A; Vieira, P; Delgado, M L; Araujo, L; Machado, J C; & Correia da Costa, J M (2009). '**Schistosoma haematobium and Schistosomiasis mansoni: production of an estradiol-related compound detected by ELISA.**' *Experimental parasitology*. 122(3), pp. 250-3.

Brand, et al., 2004 Brand, Martin D; Buckingham, Julie A; Esteves, Telma C; Green, Katherine; Lambert, Adrian J; Miwa, Satomi; Murphy, Michael P; Pakay, Julian L; Talbot, Darren A; Echtay, Karim S (2004). **"Mitochondrial superoxide and aging: uncoupling-protein activity and superoxide production."** Biochem Soc Symp. 2004;(71):203-13.

Brandhorst, et al., 2015 Brandhorst, Sebastian; Choi, In Young; Wei, Min; Cheng, Chia Wei; Sedrakyan, Sargis; Navarrete, Gerardo; Dubeau, Louis; Yap, Li Peng; Park, Ryan; Vinciguerra, Manlio; Di Biase, Stefano; Mirzaei, Hamed; Mirisola, Mario G; Childress, Patra; Ji, Lingyun; Groshen, Susan; Penna, Fabio; Odetti, Patrizio; Perin, Laura; Conti, Peter S; Ikeno, Yuji; Kennedy, Brian K; Cohen, Pinchas; Morgan, Todd E; Dorff, Tanya B; & Longo, Valter D (2015). '**A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan.**' *Cell metabolism*. 22(1), pp. 86-99.

Brandon, et al., 2009 Brandon, M.C., Wallace, D.C. & Baldi, P. Bioinformatics 25, 1731–1738 (2009).

Brandt, et al., 2008 Brandt, Annely; Krohne, Georg; & Grosshans, Jorg (2008). '**The farnesylated nuclear proteins KUGELKERN and LAMIN B promote aging-like phenotypes in Drosophila flies.**' *Aging cell*. 7(4), pp. 541-51.

Bratic & Larsson, 2013 Bratic, Ana; Larsson, Nils-Goran (2013). **"The role of mitochondria in aging."** J Clin Invest. 2013 Mar;123(3):951-7. doi: 10.1172/JCI64125. Epub 2013 Mar 1.

Brawley & Ferro, 1979 Brawley, J V & Ferro, A J (1979). '**Polyamine biosynthesis during germination of yeast ascospores.**' *Journal of bacteriology*. 140(2), pp. 649-54.

Breitkreutz, et al., 2008 Breitkreutz, Bobby-Joe; Stark, Chris; Reguly, Teresa; Boucher, Lorrie; Breitkreutz, Ashton; Livstone, Michael; Oughtred, Rose; Lackner, Daniel H; Bahler, Jurg; Wood, Valerie; Dolinski, Kara; & Tyers, Mike (2008). '**The BioGRID Interaction Database: 2008 update.**' *Nucleic acids research*. 36(Database issue), pp. D637-40.

Brenkman & Burgering, 2003 Brenkman, Arjan B & Burgering, Boudewijn M T (2003). '**FoxO3a eggs on fertility and aging.**' *Trends in molecular medicine*. 9(11), pp. 464-7.

Brett & Rando, 2014 Brett, Jamie O & Rando, Thomas A (2014). '**Alive and well? Exploring disease by studying lifespan.**' *Current opinion in genetics & development*. 26, pp. 33-40.

Brigui, et al., 1990 Brigui, N; Le Bourg, E; & Medioni, J (1990). '**Conditioned suppression of the proboscis-extension response in young, middle-aged, and old Drosophila melanogaster flies: acquisition and extinction.**' *Journal of comparative psychology (Washington, D.C. : 1983)*. 104(3), pp. 289-96.

Brown, et al., 2010 Brown, C Randell; Hung, Guo-Chiuan; Dunton, Danielle; & Chiang, Hui-Ling (2010). '**The TOR complex 1 is distributed in endosomes and in retrograde vesicles that form from the vacuole membrane and plays an important role in the vacuole import and degradation pathway.**' *The Journal of biological chemistry*. 285(30), pp. 23359-70.

Brown & Jurisica, 2007 Brown, Kevin R & Jurisica, Igor (2007). '**Unequal evolutionary conservation of human protein interactions in interologous networks.**' *Genome biology*. 8(5), pp. R95.

Buaas, et al., 2004 Buaas, F William; Kirsh, Andrew L; Sharma, Manju; McLean, Derek J; Morris, Jamie L; Griswold, Michael D; de Rooij, Dirk G; & Braun, Robert E (2004). '**Plzf is required in adult male germ cells for stem cell self-renewal.**' *Nature genetics*. 36(6), pp. 647-52.

Buckland & Bodger, 2008 Buckland, A & Bodger, K (2008). '**The cost-utility of high dose oral mesalazine for moderately active ulcerative colitis.**' *Alimentary pharmacology & therapeutics*. 28(11-12), pp. 1287-96.

Budovsky, et al., 2007 Budovsky, Arie; Abramovich, Amir; Cohen, Raphael; Chalifa-Caspi, Vered; Fraifeld, Vadim (2007). **"Longevity network: construction and implications."** Mech Ageing Dev. 2007 Jan;128(1):117-24. Epub 2006 Nov 20.

Budovsky, et al., 2013 Budovsky, Arie; Craig, Thomas; Wang, Jingwei; Tacutu, Robi; Csordas, Attila; Lourenco, Joana; Fraifeld, Vadim E; & de Magalhaes, Joao Pedro (2013). '**LongevityMap: a database of human genetic variants associated with longevity.**' *Trends in genetics : TIG*. 29(10), pp. 559-60.

Buffenstein, 2008 Buffenstein, Rochelle (2008). '**Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species.**' *Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology*. 178(4), pp. 439-45.

Bulterijs, 2011 Bulterijs, Sven (2011). '**Metformin as a geroprotector.**' *Rejuvenation research*. 14(5), pp. 469-82.

Bulterijs, et al., 2015 Bulterijs, Sven; Hull, Raphaella S; Bjork, Victor C E; & Roy, Avi G (2015). '**It is time to classify biological aging as a disease.**' *Frontiers in genetics*. 6, pp. 205.

Burdge, et al., 2007 Burdge, Graham C; Hanson, Mark A; Slater-Jefferies, Jo L; & Lillycrop, Karen A (2007). '**Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life?**' *The British journal of nutrition*. 97(6), pp. 1036-46.

Burns, et al., 2015 Burns, Terry C; Li, Matthew D; Mehta, Swapnil; Awad, Ahmed J; & Morgan, Alexander A (2015). '**Mouse models rarely mimic the transcriptome of human neurodegenerative diseases: A systematic bioinformatics-based critique of preclinical models.**' *European journal of pharmacology*. 759, pp. 101-17.

Byun, et al., 1995 Byun, D S; Venkatraman, J T; Yu, B P; & Fernandes, G (1995). '**Modulation of antioxidant activities and immune response by food restriction in aging Fisher-344 rats.**' *Aging (Milan, Italy)*. 7(1), pp. 40-8.

Cabelof, et al., 2003 Cabelof, Diane C; Yanamadala, Sunitha; Raffoul, Julian J; Guo, ZhongMao; Soofi, Abdulsalam; & Heydari, Ahmad R (2003). '**Caloric restriction promotes genomic stability by induction of base excision repair and reversal of its age-related decline.**' *DNA repair*. 2(3), pp. 295-307.

Caldas & Vinga, 2014 Caldas, Jose & Vinga, Susana (2014). '**Global meta-analysis of transcriptomics studies.**' *PloS one*. 9(2), pp. e89318.

Callahan, et al., 2015 Callahan, Alison; Cifuentes, Juan Jose; & Dumontier, Michel (2015). '**An evidence-based approach to identify aging-related genes in Caenorhabditis elegans.**' *BMC bioinformatics*. 16, pp. 40.

Cao, et al., 2010 Cao, Kajia; Chen-Plotkin, Alice S; Plotkin, Joshua B; & Wang, Li-San (2010). '**Age-correlated gene expression in normal and neurodegenerative human brain tissues.**' *PloS one*. 5(9).

Cao, et al., 2013 Cao, Kajia; Ryvkin, Paul; Hwang, Yih-Chii; Johnson, F Brad; & Wang, Li-San (2013). '**Analysis of nonlinear gene expression progression reveals extensive pathway and age-specific transitions in aging human brains.**' *PloS one*. 8(10), pp. e74578.

Caro, et al., 2009 Caro, Pilar; Gomez, Jose; Sanchez, Ines; Naudi, Alba; Ayala, Victoria; Lopez-Torres, Monica; Pamplona, Reinald; & Barja, Gustavo (2009). '**Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria.**' *Rejuvenation research*. 12(6), pp. 421-34.

Carone, et al., 2010 Carone, Benjamin R; Fauquier, Lucas; Habib, Naomi; Shea, Jeremy M; Hart, Caroline E; Li, Ruowang; Bock, Christoph; Li, Chengjian; Gu, Hongcang; Zamore, Phillip D; Meissner, Alexander; Weng, Zhiping; Hofmann, Hans A; Friedman, Nir; & Rando, Oliver J (2010). '**Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals.**' *Cell*. 143(7), pp. 1084-96.

Carrano, et al., 2009 Carrano, Andrea C; Liu, Zheng; Dillin, Andrew; & Hunter, Tony (2009). '**A conserved ubiquitination pathway determines longevity in response to diet restriction.**' *Nature*. 460(7253), pp. 396-9.

Castrillon, et al., 2003 Castrillon, Diego H; Miao, Lili; Kollipara, Ramya; Horner, James W; & DePinho, Ronald A (2003). '**Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a.**' *Science (New York, N.Y.)*. 301(5630), pp. 215-8.

Cavallini, et al., 2008 Cavallini, Gabriella; Donati, Alessio; Gori, Zina; Bergamini, Ettore (2008). **"Towards an understanding of the anti-aging mechanism of caloric restriction."** Curr Aging Sci. 2008 Mar;1(1):4-9.

Celeste, et al., 2002 Celeste, Arkady; Petersen, Simone; Romanienko, Peter J; Fernandez-Capetillo, Oscar; Chen, Hua Tang; Sedelnikova, Olga A; Reina-San-Martin, Bernardo; Coppola, Vincenzo; Meffre, Eric; Difilippantonio, Michael J; Redon, Christophe; Pilch, Duane R; Olaru, Alexandru; Eckhaus, Michael; Camerini-Otero, R Daniel; Tessarollo, Lino; Livak, Ferenc; Manova, Katia; Bonner, William M; Nussenzweig, Michel C; Nussenzweig, Andre (2002). **"Genomic instability in mice lacking histone H2AX."** Science. 2002 May 3;296(5569):922-7. Epub 2002 Apr 4.

Ceol, et al., 2010 Ceol, Arnaud; Chatr Aryamontri, Andrew; Licata, Luana; Peluso, Daniele; Briganti, Leonardo; Perfetto, Livia; Castagnoli, Luisa; & Cesareni, Gianni (2010). '**MINT, the molecular interaction database: 2009 update.**' *Nucleic acids research*. 38(Database issue), pp. D532-9.

Chambers, et al., 2007 Chambers, Stuart M; Shaw, Chad A; Gatza, Catherine; Fisk, C Joseph; Donehower, Lawrence A; & Goodell, Margaret A (2007). '**Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation.**' *PLoS biology*. 5(8), pp. e201.

Chan, et al., 2000 Chan, T F; Carvalho, J; Riles, L; & Zheng, X F (2000). '**A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR).**' *Proceedings of the National Academy of Sciences of the United States of America*. 97(24), pp. 13227-32.

Chattopadhyay, et al., 2009 — Chattopadhyay, Manas K; Chen, Weiping; Poy, George; Cam, Margaret; Stiles, David; & Tabor, Herbert (2009). '**Microarray studies on the genes responsive to the addition of spermidine or spermine to a Saccharomyces cerevisiae spermidine synthase mutant.**' *Yeast (Chichester, England)*. 26(10), pp. 531-44.

Chen, et al., 2003 — Chen, Jichun; Astle, Clinton M; & Harrison, David E (2003). '**Hematopoietic senescence is postponed and hematopoietic stem cell function is enhanced by dietary restriction.**' *Experimental hematology*. 31(11), pp. 1097-103.

Chen, et al., 2006 — Chen, Jie; Rider, David A; Ruan, Runsheng (2006). **"Identification of valid housekeeping genes and antioxidant enzyme gene expression change in the aging rat liver."** J Gerontol A Biol Sci Med Sci. 2006 Jan;61(1):20-7.

Chen, et al., 2014 — Chen, Haiyang; Zheng, Xiaobin; Zheng, Yixian (2014). **"Age-associated loss of lamin-B leads to systemic inflammation and gut hyperplasia."** Cell. 2014 Nov 6;159(4):829-43. doi: 10.1016/j.cell.2014.10.028.

Chen & Guarente, 2007 — Chen, Danica & Guarente, Leonard (2007). '**SIR2: a potential target for calorie restriction mimetics.**' *Trends in molecular medicine*. 13(2), pp. 64-71.

Chen & Han, 2001 — Chen, Z & Han, M (2001). '**C. elegans Rb, NuRD, and Ras regulate lin-39-mediated cell fusion during vulval fate specification.**' *Current biology : CB*. 11(23), pp. 1874-9.

Cheng, et al., 2007 — Cheng, Chao; Fabrizio, Paola; Ge, Huanying; Longo, Valter D; & Li, Lei M (2007). '**Inference of transcription modification in long-live yeast strains from their expression profiles.**' *BMC genomics*. 8, pp. 219.

Cherry, et al., 2012 — Cherry, J Michael; Hong, Eurie L; Amundsen, Craig; Balakrishnan, Rama; Binkley, Gail; Chan, Esther T; Christie, Karen R; Costanzo, Maria C; Dwight, Selina S; Engel, Stacia R; Fisk, Dianna G; Hirschman, Jodi E; Hitz, Benjamin C; Karra, Kalpana; Krieger, Cynthia J; Miyasato, Stuart R; Nash, Rob S; Park, Julie; Skrzypek, Marek S; Simison, Matt; Weng, Shuai; & Wong, Edith D (2012). '**Saccharomyces Genome Database: the genomics resource of budding yeast.**' *Nucleic acids research*. 40(Database issue), pp. D700-5.

Cheung, et al., 2000 — Cheung, P C; Salt, I P; Davies, S P; Hardie, D G; & Carling, D (2000). '**Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding.**' *The Biochemical journal*. 346 Pt 3, pp. 659-69.

Chiba, et al., 2007 — Chiba, T; Yamaza, H; Shimokawa, I (2007). **"Role of insulin and growth hormone/insulin-like growth factor-I signaling in lifespan extension: rodent longevity models for studying aging and calorie restriction."** Curr Genomics. 2007 Nov;8(7):423-8. doi: 10.2174/138920207783591726.

Choi, et al., 2016 — Choi, Ae-Ran; Kim, Ju-Hwa; Woo, Yeon Hwa; Cheon, Ji Hyun; Kim, Hyung Sik; & Yoon, Sungpil (2016). '**Co-treatment of LY294002 or MK-2206 with AZD5363 Attenuates AZD5363-induced Increase in the Level of Phosphorylated AKT.**' *Anticancer research*. 36(11), pp. 5849-5858.

Christiansen, et al., 2016 — Christiansen, Lene; Lenart, Adam; Tan, Qihua; Vaupel, James W; Aviv, Abraham; McGue, Matt; & Christensen, Kaare (2016). '**DNA methylation age is associated with mortality in a longitudinal Danish twin study.**' *Aging cell*. 15(1), pp. 149-54.

Christley, et al., 2009 — Christley, Scott; Lu, Yiming; Li, Chen; & Xie, Xiaohui (2009). '**Human genomes as email attachments.**' *Bioinformatics (Oxford, England)*. 25(2), pp. 274-5.

Chuang, et al., 2007 — Chuang, P Y; Yu, Q; Fang, W; Uribarri, J; & He, J C (2007). '**Advanced glycation endproducts induce podocyte apoptosis by activation of the FOXO4 transcription factor.**' *Kidney international*. 72(8), pp. 965-76.

Clark, et al., 2014 — Clark, Neil R; Hu, Kevin S; Feldmann, Axel S; Kou, Yan; Chen, Edward Y; Duan, Qiaonan; & Ma'ayan, Avi (2014). '**The characteristic direction: a geometrical approach to identify differentially expressed genes.**' *BMC bioinformatics*. 15, pp. 79.

Clark, et al., 2015 — Clark, Neil R; Szymkiewicz, Maciej; Wang, Zichen; Monteiro, Caroline D; Jones, Matthew R; & Ma'ayan, Avi (2015). '**Principal Angle Enrichment Analysis (PAEA): Dimensionally Reduced Multivariate Gene Set Enrichment Analysis Tool.**' *Proceedings. IEEE International Conference on Bioinformatics and Biomedicine*. 2015, pp. 256-262.

Clark & All, 2013 Neil R. Clark, Kevin Hu, Edward Y. Chen, Qioanan Duan, Avi Ma`ayan (2013) '**Characteristic Direction Approach to Identify Differentially Expressed Genes.**' arXiv:1307.8366

Clark & Ma ayan, 2011 Clark, Neil R & Ma'ayan, Avi (2011). '**Introduction to statistical methods for analyzing large data sets: gene-set enrichment analysis.**' *Science signaling*. 4(190), pp. tr4.

Clifton, 2010 Clifton, Tony (2010b) '**Oldest living rat! attained 47 months of age by water restriction.**', Available from: <http://www.youtube.com/watch?v=skLVAQgWx60>

Cline, et al., 2007 Cline, Melissa S; Smoot, Michael; Cerami, Ethan; Kuchinsky, Allan; Landys, Nerius; Workman, Chris; Christmas, Rowan; Avila-Campilo, Iliana; Creech, Michael; Gross, Benjamin; Hanspers, Kristina; Isserlin, Ruth; Kelley, Ryan; Killcoyne, Sarah; Lotia, Samad; Maere, Steven; Morris, John; Ono, Keiichiro; Pavlovic, Vuk; Pico, Alexander R; Vailaya, Aditya; Wang, Peng-Liang; Adler, Annette; Conklin, Bruce R; Hood, Leroy; Kuiper, Martin; Sander, Chris; Schmulevich, Ilya; Schwikowski, Benno; Warner, Guy J; Ideker, Trey; & Bader, Gary D (2007). '**Integration of biological networks and gene expression data using Cytoscape.**' *Nature protocols*. 2(10), pp. 2366-82.

Cloud, 2012 '**1000 Genomes Project data available on Amazon Cloud.**' NIH press release, 29 March 2012.

Colak, et al., 2013 Colak, Dilek; Nofal, Asmaa; Albakheet, Albandary; Nirmal, Maimoona; Jeprel, Hatim; Eldali, Abdelmoneim; Al-Tweigeri, Taher; Tulbah, Asma; Ajarim, Dahish; Malik, Osama Al; Inan, Mehmet S; Kaya, Namik; Park, Ben H; & Bin Amer, Suad M (2013). '**Age-specific gene expression signatures for breast tumors and cross-species conserved potential cancer progression markers in young women.**' *PloS one*. 8(5), pp. e63204.

Colman, et al., 2009 Colman, Ricki J; Anderson, Rozalyn M; Johnson, Sterling C; Kastman, Erik K; Kosmatka, Kristopher J; Beasley, T Mark; Allison, David B; Cruzen, Christina; Simmons, Heather A; Kemnitz, Joseph W; & Weindruch, Richard (2009). '**Caloric restriction delays disease onset and mortality in rhesus monkeys.**' *Science (New York, N.Y.)*. 325(5937), pp. 201-4.

Colomina, et al., 2003 Colomina, Neus; Liu, Yuhui; Aldea, Marti; & Gari, Eloi (2003). '**TOR regulates the subcellular localization of Ime1, a transcriptional activator of meiotic development in budding yeast.**' *Molecular and cellular biology*. 23(20), pp. 7415-24.

Comfort, 1960 Comfort, A. (1960) '**Discussion session. I. Definition and universality of aging.**' pp. 3-13 in B. L. Strehler (ed.), The Biology of Aging. AIBS, Washington, DC.

Comfort, 1963 Comfort, A (1963). '**Effect of delayed and resumed growth on the longevity of fish (Lebistes reticulatus, Peters) in captivity.**, *Gerontologia*, 49, pp.150-5.

Coschigano, et al., 2000 Coschigano, K T; Clemmons, D; Bellush, L L; & Kopchick, J J (2000). '**Assessment of growth parameters and life span of GHR/BP gene-disrupted mice.**' *Endocrinology*. 141(7), pp. 2608-13.

Coschigano, et al., 2003 Coschigano, Karen T; Holland, Amy N; Riders, Markus E; List, Edward O; Flyvbjerg, Allan; & Kopchick, John J (2003). '**Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span.**' *Endocrinology*. 144(9), pp. 3799-810.

Costanzo, et al., 2010 — Costanzo, Michael; Baryshnikova, Anastasia; Bellay, Jeremy; Kim, Yungil; Spear, Eric D; Sevier, Carolyn S; Ding, Huiming; Koh, Judice L Y; Toufighi, Kiana; Mostafavi, Sara; Prinz, Jeany; St Onge, Robert P; VanderSluis, Benjamin; Makhnevych, Taras; Vizeacoumar, Franco J; Alizadeh, Solmaz; Bahr, Sondra; Brost, Renee L; Chen, Yiqun; Cokol, Murat; Deshpande, Raamesh; Li, Zhijian; Lin, Zhen-Yuan; Liang, Wendy; Marback, Michaela; Paw, Jadine; San Luis, Bryan-Joseph; Shuteriqi, Ermira; Tong, Amy Hin Yan; van Dyk, Nydia; Wallace, Iain M; Whitney, Joseph A; Weirauch, Matthew T; Zhong, Guoqing; Zhu, Hongwei; Houry, Walid A; Brudno, Michael; Ragibizadeh, Sasan; Papp, Balazs; Pal, Csaba; Roth, Frederick P; Giaever, Guri; Nislow, Corey; Troyanskaya, Olga G; Bussey, Howard; Bader, Gary D; Gingras, Anne-Claude; Morris, Quaid D; Kim, Philip M; Kaiser, Chris A; Myers, Chad L; Andrews, Brenda J; & Boone, Charles (2010). '**The genetic landscape of a cell.**' *Science (New York, N.Y.)*. 327(5964), pp. 425-31.

Craig, et al., 2015 — Craig, Thomas; Smelick, Chris; Tacutu, Robi; Wuttke, Daniel; Wood, Shona H; Stanley, Henry; Janssens, Georges; Savitskaya, Ekaterina; Moskalev, Alexey; Arking, Robert; & de Magalhaes, Joao Pedro (2015). '**The Digital Ageing Atlas: integrating the diversity of age-related changes into a unified resource.**' *Nucleic acids research*. 43(Database issue), pp. D873-8.

Croft, et al., 2011 — Croft, David; O'Kelly, Gavin; Wu, Guanming; Haw, Robin; Gillespie, Marc; Matthews, Lisa; Caudy, Michael; Garapati, Phani; Gopinath, Gopal; Jassal, Bijay; Jupe, Steven; Kalatskaya, Irina; Mahajan, Shahana; May, Bruce; Ndegwa, Nelson; Schmidt, Esther; Shamovsky, Veronica; Yung, Christina; Birney, Ewan; Hermjakob, Henning; D'Eustachio, Peter; & Stein, Lincoln (2011). '**Reactome: a database of reactions, pathways and biological processes.**' *Nucleic acids research*. 39(Database issue), pp. D691-7.

Culerrier, et al., 2016 — Culerrier, Raphael; Carraz, Maelle; Mann, Carl; & Djabali, Malek (2016). '**MSK1 triggers the expression of the INK4AB/ARF locus in oncogene-induced senescence.**' *Molecular biology of the cell*. 27(17), pp. 2726-34.

Curtis, et al., 2006 — Curtis, Rory; O'Connor, Greg; & DiStefano, Peter S (2006). '**Aging networks in Caenorhabditis elegans: AMP-activated protein kinase (aak-2) links multiple aging and metabolism pathways.**' *Aging cell*. 5(2), pp. 119-26.

Curtsinger, 2007 — Curtsinger JW (2007) **Genes, aging, and prospects for extended life span.** *Minn Med.*, 2007 Oct;90(10):41-4.

d Adda di Fagagna, et al., 2003 — d'Adda di Fagagna, Fabrizio; Reaper, Philip M; Clay-Farrace, Lorena; Fiegler, Heike; Carr, Philippa; Von Zglinicki, Thomas; Saretzki, Gabriele; Carter, Nigel P; & Jackson, Stephen P (2003). '**A DNA damage checkpoint response in telomere-initiated senescence.**' *Nature*. 426(6963), pp. 194-8.

D Angelo, et al., 2009 — D'Angelo, Maximiliano A; Raices, Marcela; Panowski, Siler H; Hetzer, Martin W (2009). **"Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells."** Cell. 2009 Jan 23;136(2):284-95. doi: 10.1016/j.cell.2008.11.037.

Dai, et al., 2016 — Dai, Chun-Hua; Chen, Ping; Li, Jian; Lan, Tin; Chen, Yong-Chang; Qian, Hai; Chen, Kang; & Li, Mei-Yu (2016). '**Co-inhibition of pol theta and HR genes efficiently synergize with cisplatin to suppress cisplatin-resistant lung cancer cells survival.**' *Oncotarget*.

Dang, et al., 2009 — Dang, Weiwei; Steffen, Kristan K; Perry, Rocco; Dorsey, Jean A; Johnson, F Brad; Shilatifard, Ali; Kaeberlein, Matt; Kennedy, Brian K; & Berger, Shelley L (2009). '**Histone H4 lysine 16 acetylation regulates cellular lifespan.**' *Nature*. 459(7248), pp. 802-7.

Davis, et al., 1983 — Davis, T A; Bales, C W; & Beauchene, R E (1983). '**Differential effects of dietary caloric and protein restriction in the aging rat.**' *Experimental gerontology*. 18(6), pp. 427-35.

de Cabo, et al., 2015 — de Cabo, Rafael; Liu, Lijuan; Ali, Ahmed; Price, Nathan; Zhang, Jing; Wang, Mingyi; Lakatta, Edward; & Irusta, Pablo M (2015). '**Serum from calorie-restricted animals delays senescence and extends the lifespan of normal human fibroblasts in vitro.**' *Aging*. 7(3), pp. 152-66.

de Carbo, et al., 2009 de Cabo, Rafael; Siendones, Emilio; Minor, Robin; Navas, Placido (2009). **"CYB5R3: a key player in aerobic metabolism and aging?"** Aging (Albany NY). 2009 Dec 29;2(1):63-8.

de Castro, et al., 2004 de Castro, Edouard; Hegi de Castro, Sarah; Johnson, Thomas E (2004). **"Isolation of long-lived mutants in Caenorhabditis elegans using selection for resistance to juglone."** Free Radic Biol Med. 2004 Jul 15;37(2):139-45.

De Cecco, et al., 2013 De Cecco, Marco; Criscione, Steven W; Peckham, Edward J; Hillenmeyer, Sara; Hamm, Eliza A; Manivannan, Jayameenakshi; Peterson, Abigail L; Kreiling, Jill A; Neretti, Nicola; & Sedivy, John M (2013). '**Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements.**' *Aging cell*. 12(2), pp. 247-56.

de Grey, 2007 de Grey, Aubrey D.N.J. (2007). '**Life Span Extension Research and Public Debate: Societal Considerations**'. *Studies in Ethics, Law, and Technology 1* (1, Article 5), doi:10.2202/1941-6008.1011, Retrieved August 7, 2011.

de Magalhaes, 1997 de Magalhães JP (2012) '**Curing Aging**', "As detailed elsewhere, the process of aging is surprisingly plastic and can be manipulated by genetic and environmental interventions." Available from <http://www.senescence.info/aging_cure.html>

de Magalhaes, 2009 de Magalhaes, Joao Pedro (2009). '**Ageing research in the post-genome era: new technologies for an old problem.**' *SEB experimental biology series*. 62, pp. 99-115.

de Magalhaes, et al., 2005 de Magalhaes, Joao Pedro; Costa, Joana; & Toussaint, Olivier (2005). '**HAGR: the Human Ageing Genomic Resources.**' *Nucleic acids research*. 33(Database issue), pp. D537-43.

de Magalhaes, et al., 2009 de Magalhaes, Joao Pedro; Curado, Joao; & Church, George M (2009). '**Meta-analysis of age-related gene expression profiles identifies common signatures of aging.**' *Bioinformatics (Oxford, England)*. 25(7), pp. 875-81.

de Magalhaes, et al., 2009a de Magalhaes, Joao Pedro; Curado, Joao; & Church, George M (2009). '**Meta-analysis of age-related gene expression profiles identifies common signatures of aging.**' *Bioinformatics (Oxford, England)*. 25(7), pp. 875-81.

de Magalhaes, et al., 2009b de Magalhaes, Joao Pedro; Budovsky, Arie; Lehmann, Gilad; Costa, Joana; Li, Yang; Fraifeld, Vadim; & Church, George M (2009). '**The Human Ageing Genomic Resources: online databases and tools for biogerontologists.**' *Aging cell*. 8(1), pp. 65-72.

de Magalhaes, et al., 2010 de Magalhaes, Joao Pedro; Finch, Caleb E; & Janssens, Georges (2010). '**Next-generation sequencing in aging research: emerging applications, problems, pitfalls and possible solutions.**' *Ageing research reviews*. 9(3), pp. 315-23.

de Magalhaes, et al., 2012 de Magalhaes, Joao Pedro; Wuttke, Daniel; Wood, Shona H; Plank, Michael; & Vora, Chintan (2012). '**Genome-environment interactions that modulate aging: powerful targets for drug discovery.**' *Pharmacological reviews*. 64(1), pp. 88-101.

de Magalhaes & Church, 2007 de Magalhaes, Joao Pedro & Church, George M (2007). '**Analyses of human-chimpanzee orthologous gene pairs to explore evolutionary hypotheses of aging.**' *Mechanisms of ageing and development*. 128(5-6), pp. 355-64.

de Magalhaes & Toussaint, 2004 de Magalhaes, Joao Pedro & Toussaint, Olivier (2004). '**How bioinformatics can help reverse engineer human aging.**' *Ageing research reviews*. 3(2), pp. 125-41.

De Marte & Enesco, 1986 De Marte, M L & Enesco, H E (1986). '**Influence of low tryptophan diet on survival and organ growth in mice.**' *Mechanisms of ageing and development*. 36(2), pp. 161-71.

Debonneuil, et al., 2013 Debonneuil, E.; & Stambler, I (2013). **Linking Researchers – Revolutionizing Aging Research with Tools and Collaboration** *h++*.

Deluty, et al., 2015 Deluty, Jennifer A; Atzmon, Gil; Crandall, Jill; Barzilai, Nir; & Milman, Sofiya (2015). '**The influence of gender on inheritance of exceptional longevity.**' *Aging*. 7(6), pp. 412-8.

Dilova, et al., 2007 Dilova, I; Easlon, E; & Lin, S-J (2007). '**Calorie restriction and the nutrient sensing signaling pathways.**' *Cellular and molecular life sciences : CMLS*. 64(6), pp. 752-67.

Dimitrakopoulou, et al., 2015 — Dimitrakopoulou, Konstantina; Vrahatis, Aristidis G; & Bezerianos, Anastasios (2015). '**Integromics network meta-analysis on cardiac aging offers robust multi-layer modular signatures and reveals micronome synergism.**' *BMC genomics*. 16, pp. 147.

Ding, et al., 2006 — Ding, Qunxing; Martin, Sarah; Dimayuga, Edgardo; Bruce-Keller, Annadora J; Keller, Jeffrey N (2006). **"LMP2 knock-out mice have reduced proteasome activities and increased levels of oxidatively damaged proteins."** Antioxid Redox Signal. 2006 Jan-Feb;8(1-2):130-5.

Ding & Shen, 2008 — Ding, Shian-Ling & Shen, Chen-Yang (2008). '**Model of human aging: recent findings on Werner's and Hutchinson-Gilford progeria syndromes.**' *Clinical interventions in aging*. 3(3), pp. 431-44.

Dues, et al., 2016 — Dues, Dylan J; Andrews, Emily K; Schaar, Claire E; Bergsma, Alexis L; Senchuk, Megan M; Van Raamsdonk, Jeremy M (2016). **"Aging causes decreased resistance to multiple stresses and a failure to activate specific stress response pathways."** Aging (Albany NY). 2016 Apr;8(4):777-95. doi: 10.18632/aging.100939.

Dunn, et al., 1997 — Dunn, S E; Kari, F W; French, J; Leininger, J R; Travlos, G; Wilson, R; & Barrett, J C (1997). '**Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice.**' *Cancer research*. 57(21), pp. 4667-72.

Economist, 2006 — Economist (2006). **"Eat less, live more, The Economist."** April 22, 2006.

Eden, et al., 2009 — Eran Eden, Roy Navon, Israel Steinfeld, Doron Lipson, and Zohar Yakhini. '**GOrilla: a tool for discovery and visualization of enriched go terms in ranked gene lists**'. BMC Bioinformatics, 10(1):48, 2009

Eisenberg, et al., 2009 — Eisenberg, Tobias; Knauer, Heide; Schauer, Alexandra; Buttner, Sabrina; Ruckenstuhl, Christoph; Carmona-Gutierrez, Didac; Ring, Julia; Schroeder, Sabrina; Magnes, Christoph; Antonacci, Lucia; Fussi, Heike; Deszcz, Luiza; Hartl, Regina; Schraml, Elisabeth; Criollo, Alfredo; Megalou, Evgenia; Weiskopf, Daniela; Laun, Peter; Heeren, Gino; Breitenbach, Michael; Grubeck-Loebenstein, Beatrix; Herker, Eva; Fahrenkrog, Birthe; Frohlich, Kai-Uwe; Sinner, Frank; Tavernarakis, Nektarios; Minois, Nadege; Kroemer, Guido; & Madeo, Frank (2009). '**Induction of autophagy by spermidine promotes longevity.**' *Nature cell biology*. 11(11), pp. 1305-14.

Eisenberg, et al., 2016 — Eisenberg, Tobias; Abdellatif, Mahmoud; Schroeder, Sabrina; Primessnig, Uwe; Stekovic, Slaven; Pendl, Tobias; Harger, Alexandra; Schipke, Julia; Zimmermann, Andreas; Schmidt, Albrecht; Tong, Mingming; Ruckenstuhl, Christoph; Dammbrueck, Christopher; Gross, Angelina S; Herbst, Viktoria; Magnes, Christoph; Trausinger, Gert; Narath, Sophie; Meinitzer, Andreas; Hu, Zehan; Kirsch, Alexander; Eller, Kathrin; Carmona-Gutierrez, Didac; Buttner, Sabrina; Pietrocola, Federico; Knittelfelder, Oskar; Schrepfer, Emilie; Rockenfeller, Patrick; Simonini, Corinna; Rahn, Alexandros; Horsch, Marion; Moreth, Kristin; Beckers, Johannes; Fuchs, Helmut; Gailus-Durner, Valerie; Neff, Frauke; Janik, Dirk; Rathkolb, Birgit; Rozman, Jan; de Angelis, Martin Hrabe; Moustafa, Tarek; Haemmerle, Guenter; Mayr, Manuel; Willeit, Peter; von Frieling-Salewsky, Marion; Pieske, Burkert; Scorrano, Luca; Pieber, Thomas; Pechlaner, Raimund; Willeit, Johann; Sigrist, Stephan J; Linke, Wolfgang A; Muhlfeld, Christian; Sadoshima, Junichi; Dengjel, Joern; Kiechl, Stefan; Kroemer, Guido; Sedej, Simon; & Madeo, Frank (2016). '**Cardioprotection and lifespan extension by the natural polyamine spermidine.**' *Nature medicine*. 22(12), pp. 1428-1438.

Eisenberg & Levanon, 2003 — Eisenberg, Eli & Levanon, Erez Y (2003). '**Preferential attachment in the protein network evolution.**' *Physical review letters*. 91(13), pp. 138701.

Eldakak, et al., 2010 — Eldakak, Amr; Rancati, Giulia; Rubinstein, Boris; Paul, Parama; Conaway, Veronica; & Li, Rong (2010). '**Asymmetrically inherited multidrug resistance transporters are recessive determinants in cellular replicative ageing.**' *Nature cell biology*. 12(8), pp. 799-805.

Ellers, et al., 2011 — Ellers, Jacintha; Ruhe, Bas; & Visser, Bertanne (2011). '**Discriminating between energetic content and dietary composition as an explanation for dietary restriction effects.**' *Journal of insect physiology*. 57(12), pp. 1670-6.

Ellisen, et al., 2002 — Ellisen, Leif W; Ramsayer, Kate D; Johannessen, Cory M; Yang, Annie; Beppu, Hideyuki; Minda, Karolina; Oliner, Jonathan D; McKeon, Frank; & Haber, Daniel A (2002). '**REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species.**' *Molecular cell*. 10(5), pp. 995-1005.

Elshorbagy, et al., 2011 — Elshorbagy, Amany K; Valdivia-Garcia, Maria; Mattocks, Dwight A L; Plummer, Jason D; Smith, A David; Drevon, Christian A; Refsum, Helga; & Perrone, Carmen E (2011). '**Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme A desaturase.**' *Journal of lipid research*. 52(1), pp. 104-12.

Emanuele, et al., 2014 — Emanuele, Enzo; Minoretti, Piercarlo; Sanchis-Gomar, Fabian; Pareja-Galeano, Helios; Yilmaz, Yusuf; Garatachea, Nuria; Lucia, Alejandro (2014). **"Can enhanced autophagy be associated with human longevity? Serum levels of the autophagy biomarker beclin-1 are increased in healthy centenarians."** Rejuvenation Res. 2014 Dec;17(6):518-24. doi: 10.1089/rej.2014.1607.

Emran, et al., 2014 — Emran, Sahar; Yang, Mingyao; He, Xiaoli; Zandveld, Jelle; & Piper, Matthew D W (2014). '**Target of rapamycin signalling mediates the lifespan-extending effects of dietary restriction by essential amino acid alteration.**' *Aging*. 6(5), pp. 390-8.

Eppinger, et al., 2010 — Eppinger, Ben; Herbert, Michael; & Kray, Jutta (2010). '**We remember the good things: Age differences in learning and memory.**' *Neurobiology of learning and memory*. 93(4), pp. 515-21.

Espada, et al., 2008 — Espada, Jesus; Varela, Ignacio; Flores, Ignacio; Ugalde, Alejandro P; Cadinanos, Juan; Pendas, Alberto M; Stewart, Colin L; Tryggvason, Karl; Blasco, Maria A; Freije, Jose M P; & Lopez-Otin, Carlos (2008). '**Nuclear envelope defects cause stem cell dysfunction in premature-aging mice.**' *The Journal of cell biology*. 181(1), pp. 27-35.

Fabris, et al., 2015 — Fabris, Fabio; Freitas, Alex; Tullet, Jennifer (2015). **"An Extensive Empirical Comparison of Probabilistic Hierarchical Classifiers in Datasets of Ageing-Related Genes."** IEEE/ACM Trans Comput Biol Bioinform. 2015 Dec 3.

Fabrizio, et al., 2010 — Fabrizio, Paola; Hoon, Shawn; Shamalnasab, Mehrnaz; Galbani, Abdulaye; Wei, Min; Giaever, Guri; Nislow, Corey; & Longo, Valter D (2010). '**Genome-wide screen in Saccharomyces cerevisiae identifies vacuolar protein sorting, autophagy, biosynthetic, and tRNA methylation genes involved in life span regulation.**' *PLoS genetics*. 6(7), pp. e1001024.

Falender, et al., 2005 — Falender, Allison E; Freiman, Richard N; Geles, Kenneth G; Lo, Kirk C; Hwang, KeumSil; Lamb, Dolores J; Morris, Patricia L; Tjian, Robert; & Richards, JoAnne S (2005). '**Maintenance of spermatogenesis requires TAF4b, a gonad-specific subunit of TFIID.**' *Genes & development*. 19(7), pp. 794-803.

Fedichev, et al., 2011 — Fedichev, P.; Vinnik, A.; & Moskalev A. (2011). '**De-novo geroprotector design.**'

Fernandes, et al., 1976 — Fernandes, G; Yunis, E J; & Good, R A (1976). '**Influence of diet on survival of mice.**' *Proceedings of the National Academy of Sciences of the United States of America*. 73(4), pp. 1279-83.

Fernandes, et al., 2016 — Fernandes, Maria; Wan, Cen; Tacutu, Robi; Barardo, Diogo; Rajput, Ashish; Wang, Jingwei; Thoppil, Harikrishnan; Thornton, Daniel; Yang, Chenhao; Freitas, Alex; de Magalhaes, Joao Pedro (2016). **"Systematic analysis of the gerontome reveals links between aging and age-related diseases."** Hum Mol Genet. 2016 Sep 4. pii: ddw307.

Ferrarini, et al., 2005 — Ferrarini, Luca; Bertelli, Luca; Feala, Jacob; McCulloch, Andrew D; Paternostro, Giovanni (2005). **"A more efficient search strategy for aging genes based on connectivity."** Bioinformatics. 2005 Feb 1;21(3):338-48. Epub 2004 Sep 3.

Feser & Tyler, 2011 — Feser, Jason & Tyler, Jessica (2011). '**Chromatin structure as a mediator of aging.**' *FEBS letters*. 585(13), pp. 2041-8.

Finch, 1990 — Finch, C. E. (1990). '**Longevity, Senescence, and the Genome.**' University of Chicago Press, Chicago.

Finch & Kirkwood, 2000 Finch, C. E. and T. B. L. Kirkwood. 2000. Chance, Development, and Aging. Oxford University Press, New York.

Fischer, 1932 Fisher RA (1932). '**Statistical methods for research workers.**' Edinburgh etc.: Oliver and Boyd. xiii p., 1 l. p.

Fischer, et al., 2011 Fischer, Steve; Brunk, Brian P; Chen, Feng; Gao, Xin; Harb, Omar S; Iodice, John B; Shanmugam, Dhanasekaran; Roos, David S; & Stoeckert, Christian J Jr (2011). '**Using OrthoMCL to assign proteins to OrthoMCL-DB groups or to cluster proteomes into new ortholog groups.**' *Current protocols in bioinformatics*. Chapter 6, pp. Unit 6.12.1-19.

Flinn, et al., 2010 Flinn, Rory J; Yan, Ying; Goswami, Sumanta; Parker, Peter J; & Backer, Jonathan M (2010). '**The late endosome is essential for mTORC1 signaling.**' *Molecular biology of the cell*. 21(5), pp. 833-41.

Flintoft, 2011 Flintoft, Louisa (2011). '**Epigenetics: Dad's diet lives on.**' *Nature reviews. Genetics*. 12(2), pp. 80.

Flurkey, et al., 2007 Flurkey K, Currer JM, Harrison DE (2007). '**The Mouse in Aging Research.**' In The Mouse in Biomedical Research 2nd Edition. Fox JG, et al, editors. *American College Laboratory Animal Medicine (Elsevier), Burlington, MA.* pp. 637–672.

Flurkey & Yuan, 2012 **'How the evolutionary theory of aging can guide us in the search for aging genes'** PMID: 22683607

Fokas, et al., 2012 Fokas, Emmanouil; Yoshimura, Michio; Prevo, Remko; Higgins, Geoff; Hackl, Wolfgang; Maira, Sauveur-Michel; Bernhard, Eric J; McKenna, W Gillies; & Muschel, Ruth J (2012). '**NVP-BEZ235 and NVP-BGT226, dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitors, enhance tumor and endothelial cell radiosensitivity.**' *Radiation oncology (London, England)*. 7, pp. 48.

Fontana, et al., 2008 Fontana, Luigi; Weiss, Edward P; Villareal, Dennis T; Klein, Samuel; & Holloszy, John O (2008). '**Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans.**' *Aging cell*. 7(5), pp. 681-7.

Fontana, et al., 2010 Fontana, Luigi; Partridge, Linda; & Longo, Valter D (2010). '**Extending healthy life span--from yeast to humans.**' *Science (New York, N.Y.)*. 328(5976), pp. 321-6.

Fontana, et al., 2014 Fontana, Luigi; Kennedy, Brian K; Longo, Valter D; Seals, Douglas; & Melov, Simon (2014). '**Medical research: treat ageing.**' *Nature*. 511(7510), pp. 405-7.

Fortney, et al., 2010 Fortney, Kristen; Kotlyar, Max; & Jurisica, Igor (2010). '**Inferring the functions of longevity genes with modular subnetwork biomarkers of Caenorhabditis elegans aging.**' *Genome biology*. 11(2), pp. R13.

Fortney, et al., 2015 Fortney, Kristen; Dobriban, Edgar; Garagnani, Paolo; Pirazzini, Chiara; Monti, Daniela; Mari, Daniela; Atzmon, Gil; Barzilai, Nir; Franceschi, Claudio; Owen, Art B; & Kim, Stuart K (2015). '**Genome-Wide Scan Informed by Age-Related Disease Identifies Loci for Exceptional Human Longevity.**' *PLoS genetics*. 11(12), pp. e1005728.

Fox, et al., 2006 Fox, James G.; Barthold, Stephen W.; Davisson, Muriel T.; Newcomer, Christian E.; Quimby, Fred W.; & Smith, Abigail L. (2006) '**The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models**' Second Edition, *Amerian College of Laboratory, Animal Medicine Series*.

Fraga & Esteller, 2007 Fraga, Mario F & Esteller, Manel (2007). '**Epigenetics and aging: the targets and the marks.**' *Trends in genetics : TIG*. 23(8), pp. 413-8.

Franceschi, et al., 2007 Franceschi, Claudio; Capri, Miriam; Monti, Daniela; Giunta, Sergio; Olivieri, Fabiola; Sevini, Federica; Panourgia, Maria Panagiota; Invidia, Laura; Celani, Laura; Scurti, Maria; Cevenini, Elisa; Castellani, Gastone C; Salvioli, Stefano (2007). **"Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans."** Mech Ageing Dev. 2007 Jan;128(1):92-105. Epub 2006 Nov 20.

Fraser, et al., 2002 Fraser, Hunter B; Hirsh, Aaron E; Steinmetz, Lars M; Scharfe, Curt; & Feldman, Marcus W (2002). '**Evolutionary rate in the protein interaction network.**' *Science (New York, N.Y.)*. 296(5568), pp. 750-2.

Frolkis, 1982 Frolkis, V. V. (1982) '**Aging and Life-Prolonging Processes.**' (Trans. N. Bobrov). Springer-Verlag,Vienna.

Froy, et al., 2008 Froy, Oren; Chapnik, Nava; Miskin, Ruth (2008). **"Relationship between calorie restriction and the biological clock: lessons from long-lived transgenic mice."** Rejuvenation Res. 2008 Apr;11(2):467-71. doi: 10.1089/rej.2008.0669.

Froy & Miskin, 2010 Froy, Oren & Miskin, Ruth (2010). '**Effect of feeding regimens on circadian rhythms: implications for aging and longevity.**' *Aging*. 2(1), pp. 7-27.

Frye & Walf, 2010 Frye, Cheryl A & Walf, Alicia A (2010). '**Progesterone enhances learning and memory of aged wildtype and progestin receptor knockout mice.**' *Neuroscience letters*. 472(1), pp. 38-42.

Fuellen, et al., 2010 Fuellen, Georg; Adjaye, James; de Grey, Aubrey; Lepperdinger, Gunter; de Magalhaes, Joao Pedro; Suhnel, Jurgen; & Wipat, Anil (2010). '**Bioinformatics in aging research: a workshop report.**' *Rejuvenation research*. 13(6), pp. 763-7.

Fuellen, et al., 2012 Fuellen, Georg; Dengjel, Jorn; Hoeflich, Andreas; Hoeijemakers, Jan; Kestler, Hans A; Kowald, Axel; Priebe, Steffen; Rebholz-Schuhmann, Dietrich; Schmeck, Bernd; Schmitz, Ulf; Stolzing, Alexandra; Suhnel, Jurgen; Wuttke, Daniel; & Vera, Julio (2012). '**Systems biology and bioinformatics in aging research: a workshop report.**' *Rejuvenation research*. 15(6), pp. 631-41.

Fuellen, et al., 2013 Fuellen, Georg; Boerries, Melanie; Busch, Hauke; de Grey, Aubrey; Hahn, Udo; Hiller, Thomas; Hoeflich, Andreas; Jansen, Ludger; Janssens, Georges E; Kaleta, Christoph; Meinema, Anne C; Schauble, Sascha; Simm, Andreas; Schofield, Paul N; Smith, Barry; Suhnel, Juergen; Vera, Julio; Wagner, Wolfgang; Wonne, Eva C; & Wuttke, Daniel (2013). '**In silico approaches and the role of ontologies in aging research.**' *Rejuvenation research*. 16(6), pp. 540-6.

Gabriel, et al., 2015 Gabriel, Diana; Roedl, Daniela; Gordon, Leslie B; Djabali, Karima (2015). **"Sulforaphane enhances progerin clearance in Hutchinson-Gilford progeria fibroblasts."** Aging Cell. 2015 Feb;14(1):78-91. doi: 10.1111/acel.12300. Epub 2014 Dec 16.

Galliot & Chera, 2010 Galliot, Brigitte & Chera, Simona (2010). '**The Hydra model: disclosing an apoptosis-driven generator of Wnt-based regeneration.**' *Trends in cell biology*. 20(9), pp. 514-23.

Gatti, et al., 2010 Gatti, Daniel M; Barry, William T; Nobel, Andrew B; Rusyn, Ivan; & Wright, Fred A (2010). '**Heading down the wrong pathway: on the influence of correlation within gene sets.**' *BMC genomics*. 11, pp. 574.

Gebre, et al., 2012 Gebre, Senetibeb; Connor, Richard; Xia, Yufeng; Jawed, Sanaa; Bush, John M; Bard, Martin; Elsalloukh, Hassan; & Tang, Fusheng (2012). '**Osh6 overexpression extends the lifespan of yeast by increasing vacuole fusion.**' *Cell cycle (Georgetown, Tex.)*. 11(11), pp. 2176-88.

Geifman & Rubin, 2012 Geifman, Nophar & Rubin, Eitan (2012). '**The age-phenome database.**' *SpringerPlus*. 1, pp. 4.

Geifman & Rubin, 2013 Geifman, Nophar & Rubin, Eitan (2013). '**The mouse age phenome knowledgebase and disease-specific inter-species age mapping.**' *PloS one*. 8(12), pp. e81114.

Geiger, et al., 2005 Geiger, Hartmut; Rennebeck, Gabriela; Van Zant, Gary (2005). **"Regulation of hematopoietic stem cell aging in vivo by a distinct genetic element."** Proc Natl Acad Sci U S A. 2005 Apr 5;102(14):5102-7. Epub 2005 Mar 23.

Gems, 2015 Gems, David (2015). '**The aging-disease false dichotomy: understanding senescence as pathology.**' *Frontiers in genetics*. 6, pp. 212.

Gems, et al., 2002 Gems, David; Pletcher, Scott; & Partridge, Linda (2002). '**Interpreting interactions between treatments that slow aging.**' *Aging cell*. 1(1), pp. 1-9.

Gems & Partridge, 2008 Gems, David; Partridge, Linda (2008). **"Stress-response hormesis and aging: "that which does not kill us makes us stronger"."** Cell Metab. 2008 Mar;7(3):200-3. doi: 10.1016/j.cmet.2008.01.001.

Geserick & Blasco, 2006 Geserick, Christoph; Blasco, Maria A (2006). **"Novel roles for telomerase in aging."** Mech Ageing Dev. 2006 Jun;127(6):579-83. Epub 2006 Mar 3.

Gilani, et al., 2009 Gilani, G Sarwar; Nimal Ratnayke, W M; Mueller, Rudolf; & Mazza, Giuseppe (2009). '**Effects of source of protein and supplementary extracted isoflavones and anthocyanins on longevity of Stroke-prone Spontaneously Hypertensive (SHRSP) rats.**' *The Journal of toxicological sciences*. 34(3), pp. 335-41.

Giunta, 2008 — Giunta, S (2008). **"Exploring the complex relations between inflammation and aging (inflamm-aging): anti-inflamm-aging remodelling of inflamm- aging, from robustness to frailty."** Inflamm Res. 2008 Dec;57(12):558-63. doi: 10.1007/s00011-008-7243-2.

Goddeeris, et al., 2003 — Goddeeris, M M; Cook-Wiens, E; Horton, W J; Wolf, H; Stoltzfus, J R; Borrusch, M; Grotewiel, M S (2003). **"Delayed behavioural aging and altered mortality in Drosophila beta integrin mutants."** Aging Cell. 2003 Oct;2(5):257-64.

Goertz, et al., 2011 — Goertz, Meredith J; Wu, Zhuoru; Gallardo, Teresa D; Hamra, F Kent; & Castrillon, Diego H (2011). '**Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis.**' *The Journal of clinical investigation*. 121(9), pp. 3456-66.

Golden & Melov, 2007 — Golden, Tamara R & Melov, Simon (2007). '**Gene expression changes associated with aging in C. elegans.**' *WormBook : the online review of C. elegans biology*. , pp. 1-12.

Gong, et al., 2015 — Gong, Huan; Qian, Hong; Ertl, Robin; Astle, Clinton M; Wang, Gang G; Harrison, David E; & Xu, Xiangru (2015). '**Histone modifications change with age, dietary restriction and rapamycin treatment in mouse brain.**' *Oncotarget*. 6(18), pp. 15882-90.

Gong, et al., 2016 — Gong, L; Sun, Q; Li, D W-C (2016). **"Sumoylation in Cellular Senescence and Aging."** Curr Mol Med. 2016 Dec 22.

Goodrick, 1978 — Goodrick, C L (1978). '**Body weight increment and length of life: the effect of genetic constitution and dietary protein.**' *Journal of gerontology*. 33(2), pp. 184-90.

Gorbunova & Seluanov, 2016 — Gorbunova, Vera; Seluanov, Andrei (2016). **"DNA double strand break repair, aging and the chromatin connection."** Mutat Res. 2016 Jun;788:2-6. doi: 10.1016/j.mrfmmm.2016.02.004. Epub 2016 Feb 15.

Gorgoulis & Halazonetis, 2010 — Gorgoulis, Vassilis G & Halazonetis, Thanos D (2010). '**Oncogene-induced senescence: the bright and dark side of the response.**' *Current opinion in cell biology*. 22(6), pp. 816-27.

Gosain & DiPietro, 2004 — Gosain, Ankush & DiPietro, Luisa A (2004). '**Aging and wound healing.**' *World journal of surgery*. 28(3), pp. 321-6.

Gourlay, et al., 2004 — Gourlay, Campbell W; Carpp, Lindsay N; Timpson, Paul; Winder, Steven J; Ayscough, Kathryn R (2004). **"A role for the actin cytoskeleton in cell death and aging in yeast."** J Cell Biol. 2004 Mar 15;164(6):803-9.

Grandison, et al., 2009 — Grandison, Richard C; Piper, Matthew D W; & Partridge, Linda (2009). '**Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila.**' *Nature*. 462(7276), pp. 1061-4.

Gray, et al., 2002 — Gray, W P; May, K; Sundstrom, L E (2002). **"Seizure induced dentate neurogenesis does not diminish with age in rats."** Neurosci Lett. 2002 Sep 27;330(3):235-8.

Green, et al., 2011 — Green, Douglas R; Galluzzi, Lorenzo; & Kroemer, Guido (2011). '**Mitochondria and the autophagy-inflammation-cell death axis in organismal aging.**' *Science (New York, N.Y.)*. 333(6046), pp. 1109-12.

Greer, et al., 2010 — Greer, Eric L; Maures, Travis J; Hauswirth, Anna G; Green, Erin M; Leeman, Dena S; Maro, Geraldine S; Han, Shuo; Banko, Max R; Gozani, Or; Brunet, Anne (2010). **"Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans."** Nature. 2010 Jul 15;466(7304):383-7. doi: 10.1038/nature09195. Epub 2010 Jun 16.

Greer, et al., 2011 — Greer, Eric L; Maures, Travis J; Ucar, Duygu; Hauswirth, Anna G; Mancini, Elena; Lim, Jana P; Benayoun, Berenice A; Shi, Yang; & Brunet, Anne (2011). '**Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans.**' *Nature*. 479(7373), pp. 365-71.

Griffith, et al., 2013 Griffith, Malachi; Griffith, Obi L; Coffman, Adam C; Weible, James V; McMichael, Josh F; Spies, Nicholas C; Koval, James; Das, Indraniel; Callaway, Matthew B; Eldred, James M; Miller, Christopher A; Subramanian, Janakiraman; Govindan, Ramaswamy; Kumar, Runjun D; Bose, Ron; Ding, Li; Walker, Jason R; Larson, David E; Dooling, David J; Smith, Scott M; Ley, Timothy J; Mardis, Elaine R; & Wilson, Richard K (2013). '**DGIdb: mining the druggable genome.**' *Nature methods*. 10(12), pp. 1209-10.

Gross, 2011 Gross, Michael (2011). '**Riding the wave of biological data.**' *Current biology : CB*. 21(6), pp. R204-6.

Grune, et al., 2004 Grune, Tilman; Jung, Tobias; Merker, Katrin; Davies, Kelvin J A (2004). **"Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during oxidative stress, aging, and disease."** Int J Biochem Cell Biol. 2004 Dec;36(12):2519-30.

Guertin & Lis, 2010 Guertin, Michael J & Lis, John T (2010). '**Chromatin landscape dictates HSF binding to target DNA elements.**' *PLoS genetics*. 6(9), pp. e1001114.

Guevara-Aguirre, et al., 2011 Guevara-Aguirre, Jaime; Balasubramanian, Priya; Guevara-Aguirre, Marco; Wei, Min; Madia, Federica; Cheng, Chia-Wei; Hwang, David; Martin-Montalvo, Alejandro; Saavedra, Jannette; Ingles, Sue; de Cabo, Rafael; Cohen, Pinchas; & Longo, Valter D (2011). '**Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans.**' *Science translational medicine*. 3(70), pp. 70ra13.

Guha, et al., 2013 Guha, Sujay; Cao, Min; Kane, Ryan M; Savino, Anthony M; Zou, Sige; & Dong, Yuqing (2013). '**The longevity effect of cranberry extract in Caenorhabditis elegans is modulated by daf-16 and osr-1.**' *Age (Dordrecht, Netherlands)*. 35(5), pp. 1559-74.

Guha, et al., 2014 Guha, Sujay; Natarajan, Ojas; Murbach, Cole G; Dinh, Jessica; Wilson, Ethan C; Cao, Min; Zou, Sige; & Dong, Yuqing (2014). '**Supplement timing of cranberry extract plays a key role in promoting Caenorhabditis elegans healthspan.**' *Nutrients*. 6(2), pp. 911-21.

Guldener, et al., 2006 Guldener, Ulrich; Munsterkotter, Martin; Oesterheld, Matthias; Pagel, Philipp; Ruepp, Andreas; Mewes, Hans-Werner; & Stumpflen, Volker (2006). '**MPact: the MIPS protein interaction resource on yeast.**' *Nucleic acids research*. 34(Database issue), pp. D436-41.

Gutteridge, 1985 Gutteridge, J M (1985). **"Age pigments and free radicals: fluorescent lipid complexes formed by iron- and copper-containing proteins."** Biochim Biophys Acta. 1985 Apr 25;834(2):144-8.

Haghighi, et al., 2014 Haghighi, Fatemeh; Xin, Yurong; Chanrion, Benjamin; O'Donnell, Anne H; Ge, Yongchao; Dwork, Andrew J; Arango, Victoria; & Mann, J John (2014). '**Increased DNA methylation in the suicide brain.**' *Dialogues in clinical neuroscience*. 16(3), pp. 430-8.

Hahn, et al., 2017 Hahn, Oliver; Gronke, Sebastian; Stubbs, Thomas M; Ficz, Gabriella; Hendrich, Oliver; Krueger, Felix; Andrews, Simon; Zhang, Qifeng; Wakelam, Michael J; Beyer, Andreas; Reik, Wolf; & Partridge, Linda (2017). '**Dietary restriction protects from age-associated DNA methylation and induces epigenetic reprogramming of lipid metabolism.**' *Genome biology*. 18(1), pp. 56.

Han, et al., 2012 Han, Yixing; Han, Dali; Yan, Zheng; Boyd-Kirkup, Jerome D; Green, Christopher D; Khaitovich, Philipp; Han, Jing-Dong J (2012). **"Stress-associated H3K4 methylation accumulates during postnatal development and aging of rhesus macaque brain."** Aging Cell. 2012 Dec;11(6):1055-64. doi: 10.1111/acel.12007. Epub 2012 Oct 18.

Han & Brunet, 2012 Han, Shuo & Brunet, Anne (2012). '**Histone methylation makes its mark on longevity.**' *Trends in cell biology*. 22(1), pp. 42-9.

Hansen, et al., 2005 Hansen, Malene; Hsu, Ao-Lin; Dillin, Andrew; Kenyon, Cynthia (2005). **"New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a Caenorhabditis elegans genomic RNAi screen."** PLoS Genet. 2005 Jul;1(1):119-28. Epub 2005 Jul 25.

Hansen, et al., 2007 Hansen, Malene; Taubert, Stefan; Crawford, Douglas; Libina, Nataliya; Lee, Seung-Jae; & Kenyon, Cynthia (2007). '**Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans.**' *Aging cell*. 6(1), pp. 95-110.

Happel, et al., 2008 Happel, Nicole; Doenecke, Detlef; Sekeri-Pataryas, Kalliope E; Sourlingas, Thomae G (2008). **"H1 histone subtype constitution and phosphorylation state of the ageing cell system of human peripheral blood lymphocytes."** Exp Gerontol. 2008 Mar;43(3):184-99. doi: 10.1016/j.exger.2007.11.008. Epub 2007 Dec 5.

Hardie, 2004 Hardie, D Grahame (2004). '**The AMP-activated protein kinase pathway--new players upstream and downstream.**' *Journal of cell science*. 117(Pt 23), pp. 5479-87.

Hardie, et al., 2003 Hardie, D Grahame; Scott, John W; Pan, David A; & Hudson, Emma R (2003). '**Management of cellular energy by the AMP-activated protein kinase system.**' *FEBS letters*. 546(1), pp. 113-20.

Hare, 2014 Hare, Glen (2014) '**Global Bioinformatics Market Will reach USD 12,542.4 million in 2020.**'' *Finances*.

Harman, 1965 Harman, D (1965). '**The free radical theory of aging: Effect of age on serum copper levels.**' *Journal of gerontology*. 20, pp. 151-3.

Harper, et al., 2006 Harper, James M; Leathers, Charles W; & Austad, Steven N (2006). '**Does caloric restriction extend life in wild mice?**' *Aging cell*. 5(6), pp. 441-9.

Harrison, 2011 David E. Harrison (2011) '**Life span as a biomarker**'.

Harrison, et al., 2014 Harrison, David E; Strong, Randy; Allison, David B; Ames, Bruce N; Astle, Clinton M; Atamna, Hani; Fernandez, Elizabeth; Flurkey, Kevin; Javors, Martin A; Nadon, Nancy L; Nelson, James F; Pletcher, Scott; Simpkins, James W; Smith, Daniel; Wilkinson, J Erby; & Miller, Richard A (2014). '**Acarbose, 17-alpha-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males.**' *Aging cell*. 13(2), pp. 273-82.

Harten, et al., 2011 Harten, Ingrid A; Zahr, Rima S; Lemire, Joan M; Machan, Jason T; Moses, Marsha A; Doiron, Robert J; Curatolo, Adam S; Rothman, Frank G; Wight, Thomas N; Toole, Bryan P; Gordon, Leslie B (2011). **"Age-dependent loss of MMP-3 in Hutchinson-Gilford progeria syndrome."** J Gerontol A Biol Sci Med Sci. 2011 Nov;66(11):1201-7. doi: 10.1093/gerona/glr137. Epub 2011 Aug 17.

Hartmann, et al., 2009 Hartmann, Nils; Reichwald, Kathrin; Lechel, Andre; Graf, Michael; Kirschner, Jeanette; Dorn, Alexander; Terzibasi, Eva; Wellner, Juliane; Platzer, Matthias; Rudolph, Karl Lenhard; Cellerino, Alessandro; & Englert, Christoph (2009). '**Telomeres shorten while Tert expression increases during ageing of the short-lived fish Nothobranchius furzeri.**' *Mechanisms of ageing and development*. 130(5), pp. 290-6.

Haus, et al., 2007 Haus, Jacob M; Carrithers, John A; Trappe, Scott W; & Trappe, Todd A (2007). '**Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle.**' *Journal of applied physiology (Bethesda, Md. : 1985)*. 103(6), pp. 2068-76.

Haw & Stein, 2012 Haw, Robin & Stein, Lincoln (2012). '**Using the reactome database.**' *Current protocols in bioinformatics*. Chapter 8, pp. Unit8.7.

Hayflick, 2007 Hayflick, Leonard (2007). '**Biological aging is no longer an unsolved problem.**' *Annals of the New York Academy of Sciences*. 1100, pp. 1-13.

Heber-Katz, et al., 2006 Heber-Katz, Ellen; Leferovich, John; Bedelbaeva, Khamilia; Gourevitch, Dmitri; & Clark, Lise (2006). '**Conjecture: Can continuous regeneration lead to immortality? Studies in the MRL mouse.**' *Rejuvenation research*. 9(1), pp. 3-9.

Hebert, et al., 2013 Hebert, Alexander S; Dittenhafer-Reed, Kristin E; Yu, Wei; Bailey, Derek J; Selen, Ebru Selin; Boersma, Melissa D; Carson, Joshua J; Tonelli, Marco; Balloon, Allison J; Higbee, Alan J; Westphall, Michael S; Pagliarini, David J; Prolla, Tomas A; Assadi-Porter, Fariba; Roy, Sushmita; Denu, John M; & Coon, Joshua J (2013). '**Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome.**' *Molecular cell*. 49(1), pp. 186-99.

Hejblum, et al., 2015 — Hejblum, Boris P; Skinner, Jason; & Thiebaut, Rodolphe (2015). '**Time-Course Gene Set Analysis for Longitudinal Gene Expression Data.**' *PLoS computational biology*. 11(6), pp. e1004310.

Hempenstall, et al., 2010 — Hempenstall, Sarah; Picchio, Lucie; Mitchell, Sharon E; Speakman, John R; & Selman, Colin (2010). '**The impact of acute caloric restriction on the metabolic phenotype in male C57BL/6 and DBA/2 mice.**' *Mechanisms of ageing and development*. 131(2), pp. 111-8.

Hennessy & Buchwald, 2005 — Hennessy, Edward J & Buchwald, Stephen L (2005). '**Synthesis of 4,5-dianilinophthalimide and related analogues for potential treatment of Alzheimer's disease via palladium-catalyzed amination.**' *The Journal of organic chemistry*. 70(18), pp. 7371-5.

Herlihy, et al., 1990 — Herlihy, J T; Stacy, C; & Bertrand, H A (1990). '**Long-term food restriction depresses serum thyroid hormone concentrations in the rat.**' *Mechanisms of ageing and development*. 53(1), pp. 9-16.

Herskind, et al., 1996 — Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, Vaupel JW (1996) **The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900.**. *Human genetics*, 97(3), pp.319-23.

Higami & Shimokawa, 2000 — Higami, Y; Shimokawa, I (2000). **"Apoptosis in the aging process."** Cell Tissue Res. 2000 Jul;301(1):125-32.

Hill, et al., 2016 — Hill, Cristal M; Fang, Yimin; Miquet, Johanna G; Sun, Liou Y; Masternak, Michal M; Bartke, Andrzej (2016). **"Long-lived hypopituitary Ames dwarf mice are resistant to the detrimental effects of high-fat diet on metabolic function and energy expenditure."** Aging Cell. 2016 Jun;15(3):509-21. doi: 10.1111/acel.12467. Epub 2016 Mar 17.

Hipkiss, 2007 — Hipkiss, Alan R (2007). '**On why decreasing protein synthesis can increase lifespan.**' *Mechanisms of ageing and development*. 128(5-6), pp. 412-4.

Ho, et al., 2009 — Ho, Joshua W K; Stefani, Maurizio; dos Remedios, Cristobal G; & Charleston, Michael A (2009). '**A model selection approach to discover age-dependent gene expression patterns using quantile regression models.**' *BMC genomics*. 10 Suppl 3, pp. S16.

Hoenicke & Zender, 2012 — Hoenicke, Lisa & Zender, Lars (2012). '**Immune surveillance of senescent cells--biological significance in cancer- and non-cancer pathologies.**' *Carcinogenesis*. 33(6), pp. 1123-6.

Holliday, 2017 — Holliday, R. (2007) '**Ageing: The Paradox of Life.**' *Springer: Dordrecht*, The Netherlands, 2007, pp 1–132.

Holloszy & Fontana, 2007 — Holloszy, John O & Fontana, Luigi (2007). '**Caloric restriction in humans.**' *Experimental gerontology*. 42(8), pp. 709-12.

Holmes, et al., 1992 — Holmes, G E; Bernstein, C; Bernstein, H (1992). **"Oxidative and other DNA damages as the basis of aging: a review."** Mutat Res. 1992 Sep;275(3-6):305-15.

Holtman, et al., 2015 — Holtman, Inge R; Raj, Divya D; Miller, Jeremy A; Schaafsma, Wandert; Yin, Zhuoran; Brouwer, Nieske; Wes, Paul D; Moller, Thomas; Orre, Marie; Kamphuis, Willem; Hol, Elly M; Boddeke, Erik W G M; & Eggen, Bart J L (2015). '**Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis.**' *Acta neuropathologica communications*. 3, pp. 31.

Hong, et al., 2010 — Hong, Seong-Eui; Heo, Hyoung-Sam; Kim, Dae Hyun; Kim, Min-Sun; Kim, Chul Hong; Lee, Jaewon; Yoo, Mi-Ae; Yu, Byung Pal; Leeuwenburgh, Christiaan; & Chung, Hae Young (2010). '**Revealing system-level correlations between aging and calorie restriction using a mouse transcriptome.**' *Age (Dordrecht, Netherlands)*. 32(1), pp. 15-30.

Honjoh, et al., 2009 — Honjoh, Sakiko; Yamamoto, Takuya; Uno, Masaharu; & Nishida, Eisuke (2009). '**Signalling through RHEB-1 mediates intermittent fasting-induced longevity in C. elegans.**' *Nature*. 457(7230), pp. 726-30.

Honjoh & Nishida, 2011 — Honjoh, Sakiko & Nishida, Eisuke (2011). '**Two sides of lifespan regulating genes: pro-longevity or anti-longevity?**' *Journal of biochemistry*. 149(4), pp. 381-8.

Horakova, et al., 1988 Horakova, M; Deyl, Z; Hausmann, J; & Macek, K (1988). '**The effect of low protein-high dextrin diet and subsequent food restriction upon life prolongation in Fischer 344 male rats.**' *Mechanisms of ageing and development*. 45(1), pp. 1-7.

Hornbeck, et al., 2004 Hornbeck, Peter V; Chabra, Indy; Kornhauser, Jon M; Skrzypek, Elzbieta; & Zhang, Bin (2004). '**PhosphoSite: A bioinformatics resource dedicated to physiological protein phosphorylation.**' *Proteomics*. 4(6), pp. 1551-61.

Horvath, 2013 Horvath, Steve (2013). '**DNA methylation age of human tissues and cell types.**' *Genome biology*. 14(10), pp. R115.

Hosack, et al., 2003 Hosack, Douglas A; Dennis, Glynn Jr; Sherman, Brad T; Lane, H Clifford; & Lempicki, Richard A (2003). '**Identifying biological themes within lists of genes with EASE.**' *Genome biology*. 4(10), pp. R70.

Howard & Sundaram, 2002 Howard, Robyn M & Sundaram, Meera V (2002). '**C. elegans EOR-1/PLZF and EOR-2 positively regulate Ras and Wnt signaling and function redundantly with LIN-25 and the SUR-2 Mediator component.**' *Genes & development*. 16(14), pp. 1815-27.

Huang, et al., 2007 Huang, Da Wei; Sherman, Brad T; Tan, Qina; Kir, Joseph; Liu, David; Bryant, David; Guo, Yongjian; Stephens, Robert; Baseler, Michael W; Lane, H Clifford; & Lempicki, Richard A (2007). '**DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists.**' *Nucleic acids research*. 35(Web Server issue), pp. W169-75.

Huang, et al., 2009 Huang, Da Wei; Sherman, Brad T; & Lempicki, Richard A (2009). '**Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists.**' *Nucleic acids research*. 37(1), pp. 1-13.

Huang, et al., 2009a Huang, Da Wei; Sherman, Brad T; & Lempicki, Richard A (2009). '**Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.**' *Nature protocols*. 4(1), pp. 44-57.

Huang, et al., 2009b Huang, Da Wei; Sherman, Brad T; & Lempicki, Richard A (2009). '**Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists.**' *Nucleic acids research*. 37(1), pp. 1-13.

Huang, et al., 2010 Huang, Haiyan; Liu, Chun-Chi; & Zhou, Xianghong Jasmine (2010). '**Bayesian approach to transforming public gene expression repositories into disease diagnosis databases.**' *Proceedings of the National Academy of Sciences of the United States of America*. 107(15), pp. 6823-8.

Huang, et al., 2011 Huang, Tao; Zhang, Jian; Xie, Lu; Dong, Xiao; Zhang, Lei; Cai, Yu-Dong; & Li, Yi-Xue (2011). '**Crosstissue coexpression network of aging.**' *Omics : a journal of integrative biology*. 15(10), pp. 665-71.

Huang, et al., 2015 Huang, Kai-Peng; Chen, Cheng; Hao, Jie; Huang, Jun-Ying; Liu, Pei-Qing; & Huang, He-Qing (2015). '**AGEs-RAGE system down-regulates Sirt1 through the ubiquitin-proteasome pathway to promote FN and TGF-beta1 expression in male rat glomerular mesangial cells.**' *Endocrinology*. 156(1), pp. 268-79.

Huerta, 2000 Michael Huerta; Florence Haseltine; Yuan Liu; Gregory Downing; & Belinda Seto (2000) '**NIH working definition of bioinformatics and computational biology.**' *Biomedical Information Science and Technology Initiative*. 17 July 2000. Retrieved 15 January 2017.

Huerta, et al., 2000 Huerta, Michael; Downing, Gregory; Haseltine, Florence; Seto, Belinda; & Yuan Liu (2000) '**NIH working definition of bioinformatics and computational biology.**" *Biomedical Information Science and Technology Initiative*. 17 July 2000. Retrieved 18 August 2012.

Hughes & Gottschling, 2012 Hughes, Adam L; Gottschling, Daniel E (2012). **"An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast."** Nature. 2012 Dec 13;492(7428):261-5. doi: 10.1038/nature11654. Epub 2012 Nov 21.

Huhne, et al., 2014 Huhne, Rolf; Thalheim, Torsten; Suhnel, Jurgen (2014). **"AgeFactDB--the JenAge Ageing Factor Database--towards data integration in ageing research."** Nucleic Acids Res. 2014 Jan;42(Database issue):D892-6. doi: 10.1093/nar/gkt1073. Epub 2013 Nov 11.

Hur & Walker, 2009 — Hur, Jae H & Walker, David W (2009). '**p53, sex, and aging: lessons from the fruit fly.**' *Aging*. 1(11), pp. 881-3.

Huttenhower & Hofmann, 2010 — Huttenhower, Curtis & Hofmann, Oliver (2010). '**A quick guide to large-scale genomic data mining.**' *PLoS computational biology*. 6(5), pp. e1000779.

Hyttinen, et al., 2014 — Hyttinen, Juha M T; Amadio, Marialaura; Viiri, Johanna; Pascale, Alessia; Salminen, Antero; & Kaarniranta, Kai (2014). '**Clearance of misfolded and aggregated proteins by aggrephagy and implications for aggregation diseases.**' *Ageing research reviews*. 18, pp. 16-28.

Ions, et al., 2013 — Ions, Laura J; Wakeling, Luisa A; Bosomworth, Helen J; Hardyman, Joy E J; Escolme, Suzanne M; Swan, Daniel C; Valentine, Ruth A; Mathers, John C; & Ford, Dianne (2013). '**Effects of Sirt1 on DNA methylation and expression of genes affected by dietary restriction.**' *Age (Dordrecht, Netherlands)*. 35(5), pp. 1835-49.

Isserlin, et al., 2011 — Isserlin, Ruth; El-Badrawi, Rashad A; & Bader, Gary D (2011). '**The Biomolecular Interaction Network Database in PSI-MI 2.5.**' *Database : the journal of biological databases and curation*. 2011, pp. baq037.

Ito, et al., 2001 — Ito, T; Chiba, T; Ozawa, R; Yoshida, M; Hattori, M; & Sakaki, Y (2001). '**A comprehensive two-hybrid analysis to explore the yeast protein interactome.**' *Proceedings of the National Academy of Sciences of the United States of America*. 98(8), pp. 4569-74.

Iwasaki, et al., 1988a — Iwasaki, K; Gleiser, C A; Masoro, E J; McMahan, C A; Seo, E J; & Yu, B P (1988). '**The influence of dietary protein source on longevity and age-related disease processes of Fischer rats.**' *Journal of gerontology*. 43(1), pp. B5-12.

Iwasaki, et al., 1988b — Iwasaki, K; Gleiser, C A; Masoro, E J; McMahan, C A; Seo, E J; & Yu, B P (1988). '**Influence of the restriction of individual dietary components on longevity and age-related disease of Fischer rats: the fat component and the mineral component.**' *Journal of gerontology*. 43(1), pp. B13-21.

Jaenisch & Bird, 2003 — Jaenisch, Rudolf & Bird, Adrian (2003). '**Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals.**' *Nature genetics*. 33 Suppl, pp. 245-54.

Jangir & Mehrotra, 2014 — Jangir, Deepak K & Mehrotra, Ranjana (2014). '**Raman spectroscopic evaluation of DNA adducts of a platinum containing anticancer drug.**' *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*. 130, pp. 386-9.

Jayapandian, et al., 2007 — Jayapandian, Magesh; Chapman, Adriane; Tarcea, V Glenn; Yu, Cong; Elkiss, Aaron; Ianni, Angela; Liu, Bin; Nandi, Arnab; Santos, Carlos; Andrews, Philip; Athey, Brian; States, David; & Jagadish, H V (2007). '**Michigan Molecular Interactions (MiMI): putting the jigsaw puzzle together.**' *Nucleic acids research*. 35(Database issue), pp. D566-71.

Jiang, et al., 2013 — Jiang, Nan; Du, Guyu; Tobias, Ethan; Wood, Jason G; Whitaker, Rachel; Neretti, Nicola; Helfand, Stephen L (2013). **"Dietary and genetic effects on age-related loss of gene silencing reveal epigenetic plasticity of chromatin repression during aging."** Aging (Albany NY). 2013 Nov;5(11):813-24.

Jo, et al., 2015 — Jo MC, Liu W, Gu L, Dang W, & Qin L (2015) '**High-throughput analysis of yeast replicative aging using a microfluidic system**', *Proceedings of the National Academy of Sciences of the United States of America*. [PMID:26170317]

John, et al., 2007 — John, George B; Shirley, Lane J; Gallardo, Teresa D; & Castrillon, Diego H (2007). '**Specificity of the requirement for Foxo3 in primordial follicle activation.**' *Reproduction (Cambridge, England)*. 133(5), pp. 855-63.

John, et al., 2008 — John, George B; Gallardo, Teresa D; Shirley, Lane J; & Castrillon, Diego H (2008). '**Foxo3 is a PI3K-dependent molecular switch controlling the initiation of oocyte growth.**' *Developmental biology*. 321(1), pp. 197-204.

Johnson, et al., 2012 — Johnson, Adiv A; Akman, Kemal; Calimport, Stuart R G; Wuttke, Daniel; Stolzing, Alexandra; & de Magalhaes, Joao Pedro (2012). '**The role of DNA methylation in aging, rejuvenation, and age-related disease.**' *Rejuvenation research*. 15(5), pp. 483-94.

Johnson & Johnson, 2014 Johnson, Jay E & Johnson, F Brad (2014). '**Methionine restriction activates the retrograde response and confers both stress tolerance and lifespan extension to yeast, mouse and human cells.**' *PloS one*. 9(5), pp. e97729.

Jolly, et al., 2001 Jolly, C A; Muthukumar, A; Avula, C P; Troyer, D; & Fernandes, G (2001). '**Life span is prolonged in food-restricted autoimmune-prone (NZB x NZW)F(1) mice fed a diet enriched with (n-3) fatty acids.**' *The Journal of nutrition*. 131(10), pp. 2753-60.

Jung & Brack, 2014 Jung, Yunjoon & Brack, Andrew S (2014). '**Cellular mechanisms of somatic stem cell aging.**' *Current topics in developmental biology*. 107, pp. 405-38.

Kaati, et al., 2007 Kaati, Gunnar; Bygren, Lars Olov; Pembrey, Marcus; & Sjostrom, Michael (2007). '**Transgenerational response to nutrition, early life circumstances and longevity.**' *European journal of human genetics : EJHG*. 15(7), pp. 784-90.

Kabil, et al., 2011 Kabil, Hadise; Kabil, Omer; Banerjee, Ruma; Harshman, Lawrence G; & Pletcher, Scott D (2011). '**Increased transsulfuration mediates longevity and dietary restriction in Drosophila.**' *Proceedings of the National Academy of Sciences of the United States of America*. 108(40), pp. 16831-6.

Kaeberlein, et al., 1999 Kaeberlein, M; McVey, M; & Guarente, L (1999). '**The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms.**' *Genes & development*. 13(19), pp. 2570-80.

Kaeberlein, et al., 2002 Kaeberlein, Matt; Jegalian, Beatrice; McVey, Mitch (2002). **"AGEID: a database of aging genes and interventions."** Mech Ageing Dev. 2002 Apr 30;123(8):1115-9.

Kaeberlein, et al., 2007 Kaeberlein, Matt; Burtner, Christopher R; & Kennedy, Brian K (2007). '**Recent developments in yeast aging.**' *PLoS genetics*. 3(5), pp. e84.

Kahana, et al., 2010 Kahana, Smadar; Pnueli, Lilach; Kainth, Pinay; Sassi, Holly E; Andrews, Brenda; & Kassir, Yona (2010). '**Functional dissection of IME1 transcription using quantitative promoter-reporter screening.**' *Genetics*. 186(3), pp. 829-41.

Kahn, 2011 Kahn, Scott D (2011). '**On the future of genomic data.**' *Science (New York, N.Y.)*. 331(6018), pp. 728-9.

Kakinuma, et al., 1981 Kakinuma, Y; Ohsumi, Y; & Anraku, Y (1981). '**Properties of H+-translocating adenosine triphosphatase in vacuolar membranes of SAccharomyces cerevisiae.**' *The Journal of biological chemistry*. 256(21), pp. 10859-63.

Kalous & Drahota, 1996 Kalous, M; Drahota, Z (1996). **"The role of mitochondria in aging."** Physiol Res. 1996;45(5):351-9.

Kaneko, et al., 2010 Kaneko G, Yoshinaga T, Yanagawa Y, Ozaki Y, Tsukamoto K, & Watabe S (2010). '**Calorie restriction-induced maternal longevity is transmitted to their daughters in a rotifer**'. *Functional Ecology*, 25(1) pp. 209-216 [DOI: 10.1111/j.1365-2435.2010.01773.x]

Kang, et al., 2011 Kang, Hyo Jung; Kawasawa, Yuka Imamura; Cheng, Feng; Zhu, Ying; Xu, Xuming; Li, Mingfeng; Sousa, Andre M M; Pletikos, Mihovil; Meyer, Kyle A; Sedmak, Goran; Guennel, Tobias; Shin, Yurae; Johnson, Matthew B; Krsnik, Zeljka; Mayer, Simone; Fertuzinhos, Sofia; Umlauf, Sheila; Lisgo, Steven N; Vortmeyer, Alexander; Weinberger, Daniel R; Mane, Shrikant; Hyde, Thomas M; Huttner, Anita; Reimers, Mark; Kleinman, Joel E; & Sestan, Nenad (2011). '**Spatio-temporal transcriptome of the human brain.**' *Nature*. 478(7370), pp. 483-9.

Kang, et al., 2011b Kang, Tae-Won; Yevsa, Tetyana; Woller, Norman; Hoenicke, Lisa; Wuestefeld, Torsten; Dauch, Daniel; Hohmeyer, Anja; Gereke, Marcus; Rudalska, Ramona; Potapova, Anna; Iken, Marcus; Vucur, Mihael; Weiss, Siegfried; Heikenwalder, Mathias; Khan, Sadaf; Gil, Jesus; Bruder, Dunja; Manns, Michael; Schirmacher, Peter; Tacke, Frank; Ott, Michael; Luedde, Tom; Longerich, Thomas; Kubicka, Stefan; & Zender, Lars (2011). '**Senescence surveillance of pre-malignant hepatocytes limits liver cancer development.**' *Nature*. 479(7374), pp. 547-51.

Kapahi, 2010 Kapahi, Pankaj (2010). **"Protein synthesis and the antagonistic pleiotropy hypothesis of aging."** Adv Exp Med Biol. 2010;694:30-7.

Kapahi, et al., 2004 Kapahi, Pankaj; Zid, Brian M; Harper, Tony; Koslover, Daniel; Sapin, Viveca; & Benzer, Seymour (2004). '**Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway.**' *Current biology : CB*. 14(10), pp. 885-90.

Karady, et al., 2013 Karady, Ido; Frumkin, Anna; Dror, Shiran; Shemesh, Netta; Shai, Nadav; & Ben-Zvi, Anat (2013). '**Using Caenorhabditis elegans as a model system to study protein homeostasis in a multicellular organism.**' *Journal of visualized experiments : JoVE*. (82), pp. e50840.

Karelus & Nelson, 1992 Karelus, K & Nelson, J F (1992). '**Aging impairs estrogenic suppression of hypothalamic proopiomelanocortin messenger ribonucleic acid in the mouse.**' *Neuroendocrinology*. 55(6), pp. 627-33.

Kaundal, et al., 2006 Kaundal, Ravinder K; Shah, Kaushik K; & Sharma, Shyam S (2006). '**Neuroprotective effects of NU1025, a PARP inhibitor in cerebral ischemia are mediated through reduction in NAD depletion and DNA fragmentation.**' *Life sciences*. 79(24), pp. 2293-302.

Kaushik, et al., 2007 Kaushik, Susmita; Kiffin, Roberta; Cuervo, Ana Maria (2007). **"Chaperone-mediated autophagy and aging: a novel regulatory role of lipids revealed."** Autophagy. 2007 Jul-Aug;3(4):387-9. Epub 2007 Jul 9.

Kaushik, et al., 2015 Kaushik, Gaurav; Spenlehauer, Alice; Sessions, Ayla O; Trujillo, Adriana S; Fuhrmann, Alexander; Fu, Zongming; Venkatraman, Vidya; Pohl, Danielle; Tuler, Jeremy; Wang, Mingyi; Lakatta, Edward G; Ocorr, Karen; Bodmer, Rolf; Bernstein, Sanford I; Van Eyk, Jennifer E; Cammarato, Anthony; & Engler, Adam J (2015). '**Vinculin network-mediated cytoskeletal remodeling regulates contractile function in the aging heart.**' *Science translational medicine*. 7(292), pp. 292ra99.

Kawaguchi, et al., 2002 Kawaguchi, Takumi; Osatomi, Kiyoshi; Yamashita, Hiromi; Kabashima, Tsutomu; & Uyeda, Kosaku (2002). '**Mechanism for fatty acid "sparing" effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase.**' *The Journal of biological chemistry*. 277(6), pp. 3829-35.

Kawakami, et al., 2009 Kawakami, Kyojiro; Nakamura, Akihiro; Ishigami, Akihito; Goto, Sataro; Takahashi, Ryoya (2009). **"Age-related difference of site-specific histone modifications in rat liver."** Biogerontology. 2009 Aug;10(4):415-21. doi: 10.1007/s10522-008-9176-0. Epub 2008 Sep 24.

Kawakami, et al., 2012 Kawakami, Kyojiro; Nakamura, Akihiro; & Goto, Sataro (2012). '**Dietary restriction increases site-specific histone H3 acetylation in rat liver: possible modulation by sirtuins.**' *Biochemical and biophysical research communications*. 418(4), pp. 836-40.

Kemnitz, 2011 Kemnitz, Joseph W (2011). '**Calorie restriction and aging in nonhuman primates.**' *ILAR journal*. 52(1), pp. 66-77.

Kenyon, 2010 Kenyon, Cynthia J (2010). '**The genetics of ageing.**' *Nature*. 464(7288), pp. 504-12.

Keshava Prasad, et al., 2009 Keshava Prasad, T S; Goel, Renu; Kandasamy, Kumaran; Keerthikumar, Shivakumar; Kumar, Sameer; Mathivanan, Suresh; Telikicherla, Deepthi; Raju, Rajesh; Shafreen, Beema; Venugopal, Abhilash; Balakrishnan, Lavanya; Marimuthu, Arivusudar; Banerjee, Sutopa; Somanathan, Devi S; Sebastian, Aimy; Rani, Sandhya; Ray, Somak; Harrys Kishore, C J; Kanth, Sashi; Ahmed, Mukhtar; Kashyap, Manoj K; Mohmood, Riaz; Ramachandra, Y L; Krishna, V; Rahiman, B Abdul; Mohan, Sujatha; Ranganathan, Prathibha; Ramabadran, Subhashri; Chaerkady, Raghothama; & Pandey, Akhilesh (2009). '**Human Protein Reference Database--2009 update.**' *Nucleic acids research*. 37(Database issue), pp. D767-72.

Kim, 2007 Kim, Stuart K (2007). '**Common aging pathways in worms, flies, mice and humans.**' *The Journal of experimental biology*. 210(Pt 9), pp. 1607-12.

Kim, et al., 1999 Kim, S; Benguria, A; Lai, C Y; & Jazwinski, S M (1999). '**Modulation of life-span by histone deacetylase genes in Saccharomyces cerevisiae.**' *Molecular biology of the cell*. 10(10), pp. 3125-36.

Kim, et al., 2008 Kim, Wan Kyu; Krumpelman, Chase; & Marcotte, Edward M (2008). '**Inferring mouse gene functions from genomic-scale data using a combined functional network/classification strategy.**' *Genome biology*. 9 Suppl 1, pp. S5.

Kim, et al., 2011 Kim, Eun Bae; Fang, Xiaodong; Fushan, Alexey A; Huang, Zhiyong; Lobanov, Alexei V; Han, Lijuan; Marino, Stefano M; Sun, Xiaoqing; Turanov, Anton A; Yang, Pengcheng; Yim, Sun Hee; Zhao, Xiang; Kasaikina, Marina V; Stoletzki, Nina; Peng, Chunfang; Polak, Paz; Xiong, Zhiqiang; Kiezun, Adam; Zhu, Yabing; Chen, Yuanxin; Kryukov, Gregory V; Zhang, Qiang; Peshkin, Leonid; Yang, Lan; Bronson, Roderick T; Buffenstein, Rochelle; Wang, Bo; Han, Changlei; Li, Qiye; Chen, Li; Zhao, Wei; Sunyaev, Shamil R; Park, Thomas J; Zhang, Guojie; Wang, Jun; & Gladyshev, Vadim N (2011). '**Genome sequencing reveals insights into physiology and longevity of the naked mole rat.**' *Nature*. 479(7372), pp. 223-7.

Kim, et al., 2013 Kim, You-Mi; Byun, Hae-Ok; Jee, Byul A; Cho, Hyunwoo; Seo, Yong-Hak; Kim, You-Sun; Park, Min Hi; Chung, Hae-Young; Woo, Hyun Goo; & Yoon, Gyesoon (2013). '**Implications of time-series gene expression profiles of replicative senescence.**' *Aging cell*. 12(4), pp. 622-34.

Kim, et al., 2016 Kim, Seung-Soo; Choi, Kyung-Mi; Kim, Soyoung; Park, Taesun; Cho, In-Cheol; Lee, Jae-Won; Lee, Cheol-Koo (2016). **"Whole-transcriptome analysis of mouse adipose tissue in response to short-term caloric restriction."** Mol Genet Genomics. 2016 Apr;291(2):831-47. doi: 10.1007/s00438-015-1150-3. Epub 2015 Nov 25.

Kinne-Saffran & Kinne, 1994 Kinne-Saffran, E & Kinne, R K (1994). '**Jacob Henle: the kidney and beyond.**' *American journal of nephrology*. 14(4-6), pp. 355-60.

Kinney, et al., 2001 Kinney, B A; Coschigano, K T; Kopchick, J J; Steger, R W; & Bartke, A (2001). '**Evidence that age-induced decline in memory retention is delayed in growth hormone resistant GH-R-KO (Laron) mice.**' *Physiology & behavior*. 72(5), pp. 653-60.

Kircher & Kelso, 2010 Kircher, Martin & Kelso, Janet (2010). '**High-throughput DNA sequencing--concepts and limitations.**' *BioEssays : news and reviews in molecular, cellular and developmental biology*. 32(6), pp. 524-36.

Kitamura, et al., 2006 Kitamura, Takashi; Mishina, Masayoshi; & Sugiyama, Hiroyuki (2006). '**Dietary restriction increases hippocampal neurogenesis by molecular mechanisms independent of NMDA receptors.**' *Neuroscience letters*. 393(2-3), pp. 94-6.

Kobourov, 2012 Kobourov, Stephen G. (2012), **'Spring Embedders and Force-Directed Graph Drawing Algorithms'**, arXiv:1201.3011.

Koch, et al., 2012 Koch, Carmen M; Joussen, Sylvia; Schellenberg, Anne; Lin, Qiong; Zenke, Martin; & Wagner, Wolfgang (2012). '**Monitoring of cellular senescence by DNA-methylation at specific CpG sites.**' *Aging cell*. 11(2), pp. 366-9.

Koga, et al., 2011 Koga, Hiroshi; Kaushik, Susmita; & Cuervo, Ana Maria (2011). '**Protein homeostasis and aging: The importance of exquisite quality control.**' *Ageing research reviews*. 10(2), pp. 205-15.

Kogan, et al., 2015 Kogan, Valeria; Molodtsov, Ivan; Menshikov, Leonid I; Shmookler Reis, Robert J; & Fedichev, Peter (2015). '**Stability analysis of a model gene network links aging, stress resistance, and negligible senescence.**' *Scientific reports*. 5, pp. 13589.

Kohn, 1978 Kohn, R. R. (1978) '**Principles of Mammalian Aging.**', 2nd ed. Prentice-Hall, Englewood Cliffs, NJ.

Kolata, 2012 Kolata, Gina (2012). '**Severe Diet Doesn't Prolong Life, at Least in Monkeys.**'.

Konings, et al., 2014 Konings, E; Timmers, S; Boekschoten, M V; Goossens, G H; Jocken, J W; Afman, L A; Muller, M; Schrauwen, P; Mariman, E C; & Blaak, E E (2014). '**The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men.**' *International journal of obesity (2005)*. 38(3), pp. 470-3.

Koonin & Wolf, 2010 Koonin, Eugene V & Wolf, Yuri I (2010). '**Constraints and plasticity in genome and molecular-phenome evolution.**' *Nature reviews. Genetics*. 11(7), pp. 487-98.

Kops, et al., 2002 Kops, Geert J P L; Medema, Rene H; Glassford, Janet; Essers, Marieke A G; Dijkers, Pascale F; Coffer, Paul J; Lam, Eric W-F; & Burgering, Boudewijn M T (2002). '**Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors.**' *Molecular and cellular biology*. 22(7), pp. 2025-36.

Korkushko, et al., 2011 Korkushko, O V; Khavinson, V Kh; Shatilo, V B; & Antonyk-Sheglova, I A (2011). '**Peptide geroprotector from the pituitary gland inhibits rapid aging of elderly people: results of 15-year follow-up**' *Bulletin of experimental biology and medicine*. 151(3), pp. 366-9.

Koster, 1986 Koster, J F (1986). **"[The biological basis of aging. The free radicals theory]."** Tijdschr Gerontol Geriatr. 1986 Jun;17(3):99-103.

Koubova & Guarente, 2003 Koubova, Jana & Guarente, Leonard (2003). '**How does calorie restriction work?**' *Genes & development*. 17(3), pp. 313-21.

Kouzmenko, et al., 2010 Kouzmenko, Alexander; Ohtake, Fumiaki; Fujiki, Ryoji; & Kato, Shigeaki (2010). '**Hormonal gene regulation through DNA methylation and demethylation.**' *Epigenomics*. 2(6), pp. 765-74.

Kozanitis, et al., 2010 Kozanitis, Christos; Saunders, Chris; Kruglyak, Semyon; Bafna, Vineet; & Varghese, George (2010) '**Compressing Genomic Sequence Fragments Using SlimGene.**' in Research in Computational Molecular Biology, vol. 6044 of Lecture Notes in Computer Science (Berger, B., ed.) 310–324 (Springer Berlin/Heidelberg, 2010).

Kramer, et al., 2003 Kramer, Jamie M; Davidge, Jason T; Lockyer, Joseph M; & Staveley, Brian E (2003). '**Expression of Drosophila FOXO regulates growth and can phenocopy starvation.**' *BMC developmental biology*. 3, pp. 5.

Kreienkamp, et al., 2016 Kreienkamp, Ray; Croke, Monica; Neumann, Martin A; Bedia-Diaz, Gonzalo; Graziano, Simona; Dusso, Adriana; Dorsett, Dale; Carlberg, Carsten; Gonzalo, Susana (2016). **"Vitamin D receptor signaling improves Hutchinson-Gilford progeria syndrome cellular phenotypes."** Oncotarget. 2016 May 24;7(21):30018-31. doi: 10.18632/oncotarget.9065.

Kriete & Mayo, 2009 Kriete, Andres & Mayo, Kelli L (2009). '**Atypical pathways of NF-kappaB activation and aging.**' *Experimental gerontology*. 44(4), pp. 250-5.

Krishnamurthy, et al., 2004 Krishnamurthy, Janakiraman; Torrice, Chad; Ramsey, Matthew R; Kovalev, Grigoriy I; Al-Regaiey, Khalid; Su, Lishan; & Sharpless, Norman E (2004). '**Ink4a/Arf expression is a biomarker of aging.**' *The Journal of clinical investigation*. 114(9), pp. 1299-307.

Kristiansson, et al., 2013 Kristiansson, Erik; Osterlund, Tobias; Gunnarsson, Lina; Arne, Gabriella; Larsson, D G Joakim; & Nerman, Olle (2013). '**A novel method for cross-species gene expression analysis.**' *BMC bioinformatics*. 14, pp. 70.

Kubo, et al., 1987 Kubo, C; Johnson, B C; Gajjar, A; & Good, R A (1987). '**Crucial dietary factors in maximizing life span and longevity in autoimmune-prone mice.**' *The Journal of nutrition*. 117(6), pp. 1129-35.

Kuilman, et al., 2010 Kuilman, Thomas; Michaloglou, Chrysiis; Mooi, Wolter J; Peeper, Daniel S (2010). **"The essence of senescence."** Genes Dev. 2010 Nov 15;24(22):2463-79. doi: 10.1101/gad.1971610.

Kumlien, 2017 Kumlien, Emily (2017) '**Calorie restriction lets monkeys live long and prosper.**' ScienceDirect. 17 January 2017. Retrieved 15 February 2017.

Kurdistani, et al., 2004 Kurdistani, Siavash K; Tavazoie, Saeed; & Grunstein, Michael (2004). '**Mapping global histone acetylation patterns to gene expression.**' *Cell*. 117(6), pp. 721-33.

Kwon, et al., 2007 Kwon, Suk Hyung; Kim, Sun Young; Ha, Kyoung Wook; Kang, Myung Joo; Huh, Jin Seo; Im, Tae Jong; Kim, Yong Min; Park, Young Mi; Kang, Kyoung Hoon; Lee, Sangkil; Chang, Jung Yun; Lee, Jaehwi; & Choi, Young Wook (2007). '**Pharmaceutical evaluation of genistein-loaded pluronic micelles for oral delivery.**' *Archives of pharmacal research*. 30(9), pp. 1138-43.

Kwon, et al., 2010 Kwon, Jekeun; Lee, Byungwook; Chung, Haeyoung (2010). **"Gerontome: a web-based database server for aging-related genes and analysis pipelines."** BMC Genomics. 2010 Dec 2;11 Suppl 4:S20. doi: 10.1186/1471-2164-11-S4-S20.

Lakowski & Hekimi, 1998 Lakowski, B & Hekimi, S (1998). '**The genetics of caloric restriction in Caenorhabditis elegans.**' *Proceedings of the National Academy of Sciences of the United States of America*. 95(22), pp. 13091-6.

Lamb, 2007 Lamb, Justin (2007). '**The Connectivity Map: a new tool for biomedical research.**' *Nature reviews. Cancer*. 7(1), pp. 54-60.

Lamech & Haynes, 2015 Lamech, Lilian T & Haynes, Cole M (2015). '**The unpredictability of prolonged activation of stress response pathways.**' *The Journal of cell biology*. 209(6), pp. 781-7.

Lamont & Amir, 2017 Lamont, E.W.; & Amir S. (2017). '**Circadian and Ultradian Clocks/Rhythms.**' *Reference Module in Neuroscience and Biobehavioral Psychology*. <http://dx.doi.org/10.1016/B978-0-08-045396-5.00223-2>

Lander, et al., 2001 Lander, E S, et Al (2001). '**Initial sequencing and analysis of the human genome.**' *Nature*. 409(6822), pp. 860-921.

Lanza, et al., 2008 Lanza, Ian R; Short, Daniel K; Short, Kevin R; Raghavakaimal, Sreekumar; Basu, Rita; Joyner, Michael J; McConnell, Joseph P; & Nair, K Sreekumaran (2008). '**Endurance exercise as a countermeasure for aging.**' *Diabetes*. 57(11), pp. 2933-42.

Lawler, et al., 2008 Lawler, Dennis F; Larson, Brian T; Ballam, Joan M; Smith, Gail K; Biery, Darryl N; Evans, Richard H; Greeley, Elizabeth H; Segre, Mariangela; Stowe, Howard D; & Kealy, Richard D (2008). '**Diet restriction and ageing in the dog: major observations over two decades.**' *The British journal of nutrition*. 99(4), pp. 793-805.

Le, 2015 Le, Duc-Hau (2015). '**A novel method for identifying disease associated protein complexes based on functional similarity protein complex networks.**' *Algorithms for molecular biology : AMB*. 10, pp. 14.

Le Masson, et al., 2011 Le Masson, Florent; Razak, Zak; Kaigo, Mo; Audouard, Christophe; Charry, Colette; Cooke, Howard; Westwood, J Timothy; & Christians, Elisabeth S (2011). '**Identification of heat shock factor 1 molecular and cellular targets during embryonic and adult female meiosis.**' *Molecular and cellular biology*. 31(16), pp. 3410-23.

Ledda, et al., 2001 Ledda, M; Barni, L; Altieri, L; & Pannese, E (2001). '**Changes with age in the Golgi apparatus of rabbit spinal ganglion neurons.**' *Tissue & cell*. 33(4), pp. 342-8.

Lee, et al., 1999 Lee, C K; Klopp, R G; Weindruch, R; & Prolla, T A (1999). '**Gene expression profile of aging and its retardation by caloric restriction.**' *Science (New York, N.Y.)*. 285(5432), pp. 1390-3.

Lee, et al., 2000 Lee, C K; Weindruch, R; & Prolla, T A (2000). '**Gene-expression profile of the ageing brain in mice.**' *Nature genetics*. 25(3), pp. 294-7.

Lee, et al., 2003 Lee, Jun Hee; Lee, Eunji; Park, Jeehye; Kim, Euysoo; Kim, Jaeseob; & Chung, Jongkyeong (2003). '**In vivo p53 function is indispensable for DNA damage-induced apoptotic signaling in Drosophila.**' *FEBS letters*. 550(1-3), pp. 5-10.

Lee, et al., 2007 Lee, Insuk; Li, Zhihua; & Marcotte, Edward M (2007). '**An improved, bias-reduced probabilistic functional gene network of baker's yeast, Saccharomyces cerevisiae.**' *PloS one*. 2(10), pp. e988.

Lee, et al., 2008 Lee, Youngseok; Paik, Donggi; Bang, Sunhoe; Kang, Jongkyun; Chun, Bumkoo; Lee, Seungbok; Bae, Eunkyung; Chung, Jongkyung; Kim, Jaeseob (2008). **"Loss of spastic paraplegia gene atlastin induces age-dependent death of dopaminergic neurons in Drosophila."** Neurobiol Aging. 2008 Jan;29(1):84-94. Epub 2006 Oct 9.

Lee, et al., 2009 Lee, Mihye; Paik, Sang Kyoo; Lee, Min-Jung; Kim, Yoon-Jung; Kim, Sungdae; Nahm, Minyeop; Oh, Soo-Jin; Kim, Hyun-Man; Yim, Jeongbin; Lee, C Justin; Bae, Yong Chul; Lee, Seungbok (2009). **"Drosophila Atlastin regulates the stability of muscle microtubules and is required for synapse development."** Dev Biol. 2009 Jun 15;330(2):250-62. doi: 10.1016/j.ydbio.2009.03.019. Epub 2009 Mar 31.

Lee, et al., 2010 Lee, Insuk; Lehner, Ben; Vavouri, Tanya; Shin, Junha; Fraser, Andrew G; & Marcotte, Edward M (2010). '**Predicting genetic modifier loci using functional gene networks.**' *Genome research*. 20(8), pp. 1143-53.

Lee, et al., 2014 Lee, Byung Cheon; Kaya, Alaattin; Ma, Siming; Kim, Gwansu; Gerashchenko, Maxim V; Yim, Sun Hee; Hu, Zhen; Harshman, Lawrence G; & Gladyshev, Vadim N (2014). '**Methionine restriction extends lifespan of Drosophila melanogaster under conditions of low amino-acid status.**' *Nature communications*. 5, pp. 3592.

Lee & Lee, 2008 Lee, Yae-Lim & Lee, Cheol-Koo (2008). '**Transcriptional response according to strength of calorie restriction in Saccharomyces cerevisiae.**' *Molecules and cells*. 26(3), pp. 299-307.

Lehmann, et al., 2003 Lehmann, Ordan J; Sowden, Jane C; Carlsson, Peter; Jordan, Tim; & Bhattacharya, Shomi S (2003). '**Fox's in development and disease.**' *Trends in genetics : TIG*. 19(6), pp. 339-44.

Lener, et al., 2006 Lener, Thomas; Moll, Pamela Renate; Rinnerthaler, Mark; Bauer, Johann; Aberger, Fritz; & Richter, Klaus (2006). '**Expression profiling of aging in the human skin.**' *Experimental gerontology*. 41(4), pp. 387-97.

Lengfeld, et al., 2009 Lengfeld, Tobias; Watanabe, Hiroshi; Simakov, Oleg; Lindgens, Dirk; Gee, Lydia; Law, Lee; Schmidt, Heiko A; Ozbek, Suat; Bode, Hans; & Holstein, Thomas W (2009). '**Multiple Wnts are involved in Hydra organizer formation and regeneration.**' *Developmental biology*. 330(1), pp. 186-99.

Lesne, et al., 2006 Lesne, Sylvain; Koh, Ming Teng; Kotilinek, Linda; Kayed, Rakez; Glabe, Charles G; Yang, Austin; Gallagher, Michela; & Ashe, Karen H (2006). '**A specific amyloid-beta protein assembly in the brain impairs memory.**' *Nature*. 440(7082), pp. 352-7.

Leto, et al., 1976 Leto, S; Kokkonen, G C; & Barrows, C H Jr (1976). '**Dietary protein, life-span, and biochemical variables in female mice.**' *Journal of gerontology*. 31(2), pp. 144-8.

Lewis, et al., 2008 Lewis, Michael C; Orr, Patrick T; & Frick, Karyn M (2008). '**Differential effects of acute progesterone administration on spatial and object memory in middle-aged and aged female C57BL/6 mice.**' *Hormones and behavior*. 54(3), pp. 455-62.

Li, et al., 2002 Li, Qiang; Xiao, Hengyi; Isobe, Ken-ichi (2002). **"Histone acetyltransferase activities of cAMP-regulated enhancer-binding protein and p300 in tissues of fetal, young, and old mice."** J Gerontol A Biol Sci Med Sci. 2002 Mar;57(3):B93-8.

Li, et al., 2010 Li, Yan-Hui; Dong, Meng-Qiu; & Guo, Zheng (2010). '**Systematic analysis and prediction of longevity genes in Caenorhabditis elegans.**' *Mechanisms of ageing and development*. 131(11-12), pp. 700-9.

Li, et al., 2011 Li, Yuanyuan; Daniel, Michael; & Tollefsbol, Trygve O (2011). '**Epigenetic regulation of caloric restriction in aging.**' *BMC medicine*. 9, pp. 98.

Li, et al., 2012 Li, Yu-Huan; Lu, Qiao-Ni; Wang, Hui-Qiang; Tao, Pei-Zhen; & Jiang, Jian-Dong (2012). '**Geldanamycin, a ligand of heat shock protein 90, inhibits herpes simplex virus type 2 replication both in vitro and in vivo.**' *The Journal of antibiotics*. 65(10), pp. 509-12.

Li, et al., 2013 Li, Weiguo; Chen, Lina; Li, Wan; Qu, Xiaoli; He, Weiming; He, Yuehan; Feng, Chenchen; Jia, Xu; Zhou, Yanyan; Lv, Junjie; Liang, Binhua; Chen, Binbin; & Jiang, Jing (2013). '**Unraveling the characteristics of microRNA regulation in the developmental and aging process of the human brain.**' *BMC medical genomics*. 6, pp. 55.

Li, et al., 2014 Li, Xiaotao; Moses, Robb E; Jin, Jianping; Cao, Weiguo; & Caulin, Carlos (2014). '**Stresses, aging, and age-related disorders.**' *Oxidative medicine and cellular longevity*. 2014, pp. 320564.

Li, et al., 2015 Li, Matthew D; Burns, Terry C; Kumar, Sunny; Morgan, Alexander A; Sloan, Steven A; & Palmer, Theo D (2015). '**Aging-like changes in the transcriptome of irradiated microglia.**' *Glia*. 63(5), pp. 754-67.

Li, et al., 2016 Li, Yuan; Chen, Baohui; Zou, Wei; Wang, Xin; Wu, Yanwei; Zhao, Dongfeng; Sun, Yanan; Liu, Yubing; Chen, Lianwan; Miao, Long; Yang, Chonglin; & Wang, Xiaochen (2016). '**The lysosomal membrane protein SCAV-3 maintains lysosome integrity and adult longevity.**' *The Journal of cell biology*. 215(2), pp. 167-185.

Li & Osborne, 2008 Li, Guang-Yu & Osborne, Neville N (2008). '**Oxidative-induced apoptosis to an immortalized ganglion cell line is caspase independent but involves the activation of poly(ADP-ribose)polymerase and apoptosis-inducing factor.**' *Brain research*. 1188, pp. 35-43.

Liao, et al., 2010 Liao, Chen-Yu; Rikke, Brad A; Johnson, Thomas E; Diaz, Vivian; & Nelson, James F (2010). '**Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening.**' *Aging cell*. 9(1), pp. 92-5.

Lima, et al., 2014 Lima, Maria E; Colpo, Ana C; Salgueiro, Willian G; Sardinha, Guilherme E; Avila, Daiana S; & Folmer, Vanderlei (2014). '**Ilex paraguariensis Extract Increases Lifespan and Protects Against the Toxic Effects Caused by Paraquat in Caenorhabditis elegans.**' *International journal of environmental research and public health*. 11(10), pp. 10091-104.

Lin, et al., 2002 Lin, Su-Ju; Kaeberlein, Matt; Andalis, Alex A; Sturtz, Lori A; Defossez, Pierre-Antoine; Culotta, Valeria C; Fink, Gerald R; & Guarente, Leonard (2002). '**Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration.**' *Nature*. 418(6895), pp. 344-8.

Lin, et al., 2007 Lin, Jonathan H; Li, Han; Yasumura, Douglas; Cohen, Hannah R; Zhang, Chao; Panning, Barbara; Shokat, Kevan M; Lavail, Matthew M; & Walter, Peter (2007). '**IRE1 signaling affects cell fate during the unfolded protein response.**' *Science (New York, N.Y.)*. 318(5852), pp. 944-9.

Linford, et al., 2013 Linford NJ1, Bilgir C, Ro J, Pletcher SD. '**Measurement of lifespan in Drosophila melanogaster**" J Vis Exp. 2013 Jan 7;(71). pii: 50068. doi: 10.3791/50068.

List, et al., 2011 List, Edward O; Sackmann-Sala, Lucila; Berryman, Darlene E; Funk, Kevin; Kelder, Bruce; Gosney, Elahu S; Okada, Shigeru; Ding, Juan; Cruz-Topete, Diana; & Kopchick, John J (2011). '**Endocrine parameters and phenotypes of the growth hormone receptor gene disrupted (GHR-/-) mouse.**' *Endocrine reviews*. 32(3), pp. 356-86.

Liu, et al., 2012 Liu, Qiang; Li, Hong; Chen, Huai-Hong; Wang, Jing (2012). **"[Caloric restriction suppresses endothelial cells senescence via down-regulation of NOX4 induced by HNF3gamma]."** Yi Chuan. 2012 May;34(5):573-83.

Liu, et al., 2013 Liu, Dongmei; Sartor, Maureen A; Nader, Gustavo A; Pistilli, Emidio E; Tanton, Leah; Lilly, Charles; Gutmann, Laurie; IglayReger, Heidi B; Visich, Paul S; Hoffman, Eric P; & Gordon, Paul M (2013). '**Microarray analysis reveals novel features of the muscle aging process in men and women.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 68(9), pp. 1035-44.

Loh, et al., 2012 Loh, Po-Ru; Baym, Michael; & Berger, Bonnie (2012). '**Compressive genomics.**' *Nature biotechnology*. 30(7), pp. 627-30.

Lohr, et al., 2015 Lohr, Mille; Jensen, Annie; Eriksen, Louise; Gronbaek, Morten; Loft, Steffen; & Moller, Peter (2015). '**Association between age and repair of oxidatively damaged DNA in human peripheral blood mononuclear cells.**' *Mutagenesis*. 30(5), pp. 695-700.

Long, et al., 2016 Long, Xubing; Li, Yuqing; Yang, Mengtian; Huang, Lu; Gong, Weijie; Kuang, Ersheng (2016). **"BZLF1 Attenuates Transmission of Inflammatory Paracrine Senescence in Epstein-Barr Virus-Infected Cells by Downregulating Tumor Necrosis Factor Alpha."** J Virol. 2016 Aug 12;90(17):7880-93. doi: 10.1128/JVI.00999-16. Print 2016 Sep 1.

Longo, 2009 Longo, Valter D (2009). '**Linking sirtuins, IGF-I signaling, and starvation.**' *Experimental gerontology*. 44(1-2), pp. 70-4.

Longo, et al., 2010 Longo, Valter D; Nislow, Corey; & Fabrizio, Paola (2010). '**Endosomal protein sorting and autophagy genes contribute to the regulation of yeast life span.**' *Autophagy*. 6(8), pp. 1227-8.

Longo, et al., 2012 Longo, Valter D; Shadel, Gerald S; Kaeberlein, Matt; & Kennedy, Brian (2012). '**Replicative and chronological aging in Saccharomyces cerevisiae.**' *Cell metabolism*. 16(1), pp. 18-31.

Lopez-Lluch & Navas, 2016 Lopez-Lluch, Guillermo & Navas, Placido (2016). '**Calorie restriction as an intervention in ageing.**' *The Journal of physiology*. 594(8), pp. 2043-60.

Lopez-Otin, et al., 2013 Lopez-Otin, Carlos; Blasco, Maria A; Partridge, Linda; Serrano, Manuel; & Kroemer, Guido (2013). '**The hallmarks of aging.**' *Cell*. 153(6), pp. 1194-217.

Loram & Bodnar, 2012 Loram, Jeannette & Bodnar, Andrea (2012). '**Age-related changes in gene expression in tissues of the sea urchin Strongylocentrotus purpuratus.**' *Mechanisms of ageing and development*. 133(5), pp. 338-47.

Lorenz, et al., 2009 Lorenz, David R; Cantor, Charles R; & Collins, James J (2009). '**A network biology approach to aging in yeast.**' *Proceedings of the National Academy of Sciences of the United States of America*. 106(4), pp. 1145-50.

Lu, et al., 2008 — Lu, Jiachun; Wei, Qingyi; Bondy, Melissa L; Brewster, Abenaa M; Bevers, Therese B; Yu, Tse-Kuan; Buchholz, Thomas A; Meric-Bernstam, Funda; Hunt, Kelly K; Singletary, S Eva; Wang, Li-E (2008). **"Genetic variants in the H2AFX promoter region are associated with risk of sporadic breast cancer in non-Hispanic white women aged <or=55 years."** Breast Cancer Res Treat. 2008 Jul;110(2):357-66. Epub 2007 Sep 13.

Lu, et al., 2010b — Lu, Wan-Jin; Chapo, Joseph; Roig, Ignasi; & Abrams, John M (2010). '**Meiotic recombination provokes functional activation of the p53 regulatory network.**' *Science (New York, N.Y.)*. 328(5983), pp. 1278-81.

Ludewig, et al., 2014 — Ludewig, Andreas Hanno; Klapper, Maja; & Doring, Frank (2014). '**Identifying evolutionarily conserved genes in the dietary restriction response using bioinformatics and subsequent testing in Caenorhabditis elegans.**' *Genes & nutrition*. 9(1), pp. 363.

Lugli, et al., 2015 — Lugli, Giovanni; Cohen, Aaron M; Bennett, David A; Shah, Raj C; Fields, Christopher J; Hernandez, Alvaro G; Smalheiser, Neil R (2015). **"Plasma Exosomal miRNAs in Persons with and without Alzheimer Disease: Altered Expression and Prospects for Biomarkers."** PLoS One. 2015 Oct 1;10(10):e0139233. doi: 10.1371/journal.pone.0139233. eCollection 2015.

Luszcz & Bryan, 1999 — Luszcz, M A & Bryan, J (1999). '**Toward understanding age-related memory loss in late adulthood.**' *Gerontology*. 45(1), pp. 2-9.

Lutz, et al., 1977 — Lutz, H; Konersmann, T; Ermini, M (1977). **"[Histone phosphorylation of human lymphocytes after PHA stimulation in relation to age (author's transl)]."** Aktuelle Gerontol. 1977 May;7(5):257-61.

Ma, et al., 2015 — Ma, Li; Bai, Yana; Pu, Hongquan; Gou, Faxiang; Dai, Min; Wang, Hui; He, Jie; Zheng, Tongzhang; & Cheng, Ning (2015). '**Histone Methylation in Nickel-Smelting Industrial Workers.**' *PloS one*. 10(10), pp. e0140339.

Ma, et al., 2017 — Ma, Cao; Pi, Chenchen; Yang, Yue; Lin, Lin; Shi, Yingai; Li, Yan; Li, Yulin; & He, Xu (2017). '**Nampt Expression Decreases Age-Related Senescence in Rat Bone Marrow Mesenchymal Stem Cells by Targeting Sirt1.**' *PloS one*. 12(1), pp. e0170930.

Machida, et al., 2015 — Machida, Tatsuya; Tomofuji, Takaaki; Ekuni, Daisuke; Maruyama, Takayuki; Yoneda, Toshiki; Kawabata, Yuya; Mizuno, Hirofumi; Miyai, Hisataka; Kunitomo, Muneyoshi; Morita, Manabu (2015). **"MicroRNAs in Salivary Exosome as Potential Biomarkers of Aging."** Int J Mol Sci. 2015 Sep 7;16(9):21294-309. doi: 10.3390/ijms160921294.

Machin, et al., 2004 — Machin, Felix; Paschos, Konstantinos; Jarmuz, Adam; Torres-Rosell, Jordi; Pade, Corinna; & Aragon, Luis (2004). '**Condensin regulates rDNA silencing by modulating nucleolar Sir2p.**' *Current biology : CB*. 14(2), pp. 125-30.

MacKay, et al., 2012 — MacKay, Kennen B; Lowenson, Jonathan D; & Clarke, Steven G (2012). '**Wortmannin reduces insulin signaling and death in seizure-prone Pcmt1-/- mice.**' *PloS one*. 7(10), pp. e46719.

Madeo, et al., 2014 — Madeo, Frank; Pietrocola, Federico; Eisenberg, Tobias; & Kroemer, Guido (2014). '**Caloric restriction mimetics: towards a molecular definition.**' *Nature reviews. Drug discovery*. 13(10), pp. 727-40.

Maekinen, et al., 2009 — Mäkinen, Veli; Navarro, Gonzalo; Sirén, Jouni; & Välimäki N. Niko (2009) '**Storage and Retrieval of Individual Genomes.**' in Research in Computational Molecular Biology, vol. 5541 of Lecture Notes in Computer Science (Batzoglou, S., ed.) 121–137 (Springer Berlin/Heidelberg, 2009).

Maier, et al., 2004 — Maier, Bernhard; Gluba, Wendy; Bernier, Brian; Turner, Terry; Mohammad, Khalid; Guise, Theresa; Sutherland, Ann; Thorner, Michael; & Scrable, Heidi (2004). '**Modulation of mammalian life span by the short isoform of p53.**' *Genes & development*. 18(3), pp. 306-19.

Mair, et al., 2003 — Mair, William; Goymer, Patrick; Pletcher, Scott D; & Partridge, Linda (2003). '**Demography of dietary restriction and death in Drosophila.**' *Science (New York, N.Y.)*. 301(5640), pp. 1731-3.

Mair, et al., 2011 Mair, William; Morantte, Ianessa; Rodrigues, Ana P C; Manning, Gerard; Montminy, Marc; Shaw, Reuben J; & Dillin, Andrew (2011). '**Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB.**' *Nature*. 470(7334), pp. 404-8.

Mair & Dillin, 2008 Mair, William & Dillin, Andrew (2008). '**Aging and survival: the genetics of life span extension by dietary restriction.**' *Annual review of biochemistry*. 77, pp. 727-54.

Maitra, et al., 2012 Maitra, Raj D; Kim, Jungsuk; & Dunbar, William B (2012). '**Recent advances in nanopore sequencing.**' *Electrophoresis*. 33(23), pp. 3418-28.

Majo, et al., 2008 Majo, Francois; Rochat, Ariane; Nicolas, Michael; Jaoude, Georges Abou; & Barrandon, Yann (2008). '**Oligopotent stem cells are distributed throughout the mammalian ocular surface.**' *Nature*. 456(7219), pp. 250-4.

Mak, et al., 2009 Mak, H Craig; Pillus, Lorraine; & Ideker, Trey (2009). '**Dynamic reprogramming of transcription factors to and from the subtelomere.**' *Genome research*. 19(6), pp. 1014-25.

Malavolta, et al., 2016 Malavolta, Marco; Pierpaoli, Elisa; Giacconi, Robertina; Costarelli, Laura; Piacenza, Francesco; Basso, Andrea; Cardelli, Maurizio; & Provinciali, Mauro (2016). '**Pleiotropic Effects of Tocotrienols and Quercetin on Cellular Senescence: Introducing the Perspective of Senolytic Effects of Phytochemicals.**' *Current drug targets*. 17(4), pp. 447-59.

Malloy, et al., 2006 Malloy, Virginia L; Krajcik, Rozlyn A; Bailey, Steven J; Hristopoulos, George; Plummer, Jason D; & Orentreich, Norman (2006). '**Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction.**' *Aging cell*. 5(4), pp. 305-14.

Manavalan, et al., 2013 Manavalan, Arulmani; Mishra, Manisha; Feng, Lin; Sze, Siu Kwan; Akatsu, Hiroyasu; Heese, Klaus (2013). **"Brain site-specific proteome changes in aging-related dementia."** Exp Mol Med. 2013 Sep 6;45:e39. doi: 10.1038/emm.2013.76.

Mannick, et al., 2014 Mannick, Joan B; Del Giudice, Giuseppe; Lattanzi, Maria; Valiante, Nicholas M; Praestgaard, Jens; Huang, Baisong; Lonetto, Michael A; Maecker, Holden T; Kovarik, John; Carson, Simon; Glass, David J; & Klickstein, Lloyd B (2014). '**mTOR inhibition improves immune function in the elderly.**' *Science translational medicine*. 6(268), pp. 268ra179.

Mari, 2011 Mari, D (2011). '**Role of the IGF/insulin system in longevity.**' *Minerva endocrinologica*. 36(3), pp. 181-5.

Martin-Montalvo, et al., 2013 Martin-Montalvo, Alejandro; Mercken, Evi M; Mitchell, Sarah J; Palacios, Hector H; Mote, Patricia L; Scheibye-Knudsen, Morten; Gomes, Ana P; Ward, Theresa M; Minor, Robin K; Blouin, Marie-Jose; Schwab, Matthias; Pollak, Michael; Zhang, Yongqing; Yu, Yinbing; Becker, Kevin G; Bohr, Vilhelm A; Ingram, Donald K; Sinclair, David A; Wolf, Norman S; Spindler, Stephen R; Bernier, Michel; & de Cabo, Rafael (2013). '**Metformin improves healthspan and lifespan in mice.**' *Nature communications*. 4, pp. 2192.

Masiello, et al., 1985 Masiello, P; Cubeddu, T L; Frosina, G; & Bergamini, E (1985). '**Protective effect of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase, against streptozotocin-induced diabetes.**' *Diabetologia*. 28(9), pp. 683-6.

Masoro, 1995 Masoro, E. J. (1995) '**Aging: Current concepts.**' pp. 3-21 in E. J. Masoro (ed.), Handbook of Physiology. Section 11: Aging. Oxford University Press, New York.

Masoro, 1996 Masoro, E J (1996). '**Possible mechanisms underlying the antiaging actions of caloric restriction.**' *Toxicologic pathology*. 24(6), pp. 738-41.

Masoro, 2005 Masoro, Edward J (2005). '**Overview of caloric restriction and ageing.**' *Mechanisms of ageing and development*. 126(9), pp. 913-22.

Masoro, 2007 Masoro, Edward J (2007). '**The role of hormesis in life extension by dietary restriction.**' *Interdisciplinary topics in gerontology*. 35, pp. 1-17.

Masoro, et al., 1991 Masoro, E J; Shimokawa, I; & Yu, B P (1991). '**Retardation of the aging processes in rats by food restriction.**' *Annals of the New York Academy of Sciences*. 621, pp. 337-52.

Matsumoto, et al., 2011 Matsumoto, Mitsuharu; Kurihara, Shin; Kibe, Ryoko; Ashida, Hisashi; & Benno, Yoshimi (2011). '**Longevity in mice is promoted by probiotic-induced suppression of colonic senescence dependent on upregulation of gut bacterial polyamine production.**' *PloS one*. 6(8), pp. e23652.

Mattison, et al., 2012 Mattison, Julie A; Roth, George S; Beasley, T Mark; Tilmont, Edward M; Handy, April M; Herbert, Richard L; Longo, Dan L; Allison, David B; Young, Jennifer E; Bryant, Mark; Barnard, Dennis; Ward, Walter F; Qi, Wenbo; Ingram, Donald K; & de Cabo, Rafael (2012). '**Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study.**' *Nature*. 489(7415), pp. 318-21.

Mattison, et al., 2017 Mattison, Julie A; Colman, Ricki J; Beasley, T Mark; Allison, David B; Kemnitz, Joseph W; Roth, George S; Ingram, Donald K; Weindruch, Richard; de Cabo, Rafael; & Anderson, Rozalyn M (2017). '**Caloric restriction improves health and survival of rhesus monkeys.**' *Nature communications*. 8, pp. 14063.

Mattson, et al., 2001 Mattson, M P; Duan, W; Lee, J; & Guo, Z (2001). '**Suppression of brain aging and neurodegenerative disorders by dietary restriction and environmental enrichment: molecular mechanisms.**' *Mechanisms of ageing and development*. 122(7), pp. 757-78.

Maxwell, et al., 2011 Maxwell, Patrick H; Burhans, William C; & Curcio, M Joan (2011). '**Retrotransposition is associated with genome instability during chronological aging.**' *Proceedings of the National Academy of Sciences of the United States of America*. 108(51), pp. 20376-81.

Maynard, 1962 Maynard, S. J. (1962) '**Review lectures on senescence. I. The causes of ageing.**' Proc. R. Soc. Lond. B 157: 115-127.

McCarty, et al., 2009 McCarty, Mark F; Barroso-Aranda, Jorge; & Contreras, Francisco (2009). '**The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy.**' *Medical hypotheses*. 72(2), pp. 125-8.

McCay, 1933 McCay, C M (1933). '**Is longevity compatible with optimum growth?**' *Science (New York, N.Y.)*. 77(2000), pp. 410-1.

McDowell, et al., 2004 McDowell, Susan A; McCall, Eileen; Matter, William F; Estridge, Thomas B; & Vlahos, Chris J (2004). '**Phosphoinositide 3-kinase regulates excitation-contraction coupling in neonatal cardiomyocytes.**' *American journal of physiology. Heart and circulatory physiology*. 286(2), pp. H796-805.

McGonigle, et al., 2015 McGonigle, Sharon; Chen, Zhihong; Wu, Jiayi; Chang, Paul; Kolber-Simonds, Donna; Ackermann, Karen; Twine, Natalie C; Shie, Jue-Lon; Miu, Jingzang Tao; Huang, Kuan-Chun; Moniz, George A; & Nomoto, Kenichi (2015). '**E7449: A dual inhibitor of PARP1/2 and tankyrase1/2 inhibits growth of DNA repair deficient tumors and antagonizes Wnt signaling.**' *Oncotarget*. 6(38), pp. 41307-23.

Melendez, et al., 2008 Melendez, Alicia; Hall, David H; Hansen, Malene (2008). **"Monitoring the role of autophagy in C. elegans aging."** Methods Enzymol. 2008;451:493-520. doi: 10.1016/S0076-6879(08)03229-1.

Merry, et al., 2008 Merry, Brian J; Kirk, Austin J; & Goyns, Malcolm H (2008). '**Dietary lipoic acid supplementation can mimic or block the effect of dietary restriction on life span.**' *Mechanisms of ageing and development*. 129(6), pp. 341-8.

Meshorer & Gruenbaum, 2008 Meshorer, Eran; Gruenbaum, Yosef (2008). **"Gone with the Wnt/Notch: stem cells in laminopathies, progeria, and aging."** J Cell Biol. 2008 Apr 7;181(1):9-13. doi: 10.1083/jcb.200802155. Epub 2008 Mar 31.

Metchat, et al., 2009 Metchat, Aicha; Akerfelt, Malin; Bierkamp, Christiane; Delsinne, Virginie; Sistonen, Lea; Alexandre, Henri; & Christians, Elisabeth S (2009). '**Mammalian heat shock factor 1 is essential for oocyte meiosis and directly regulates Hsp90alpha expression.**' *The Journal of biological chemistry*. 284(14), pp. 9521-8.

Michaut, et al., 2008 Michaut, Magali; Kerrien, Samuel; Montecchi-Palazzi, Luisa; Chauvat, Franck; Cassier-Chauvat, Corinne; Aude, Jean-Christophe; Legrain, Pierre; & Hermjakob, Henning (2008). '**InteroPORC: automated inference of highly conserved protein interaction networks.**' *Bioinformatics (Oxford, England)*. 24(14), pp. 1625-31.

Miller, 2001 — Miller, R A (2001). '**Biomarkers of aging: prediction of longevity by using age-sensitive T-cell subset determinations in a middle-aged, genetically heterogeneous mouse population.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 56(4), pp. B180-6

Miller, et al., 2002 — Miller, Richard A; Chang, Yayi; Galecki, Andrzej T; Al-Regaiey, Khalid; Kopchick, John J; & Bartke, Andrzej (2002). '**Gene expression patterns in calorically restricted mice: partial overlap with long-lived mutant mice.**' *Molecular endocrinology (Baltimore, Md.)*. 16(11), pp. 2657-66.

Miller, et al., 2005 — Miller, Richard A; Buehner, Gretchen; Chang, Yayi; Harper, James M; Sigler, Robert; & Smith-Wheelock, Michael (2005). '**Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance.**' *Aging cell*. 4(3), pp. 119-25.

Miller & Payne, 1968 — Miller, D S & Payne, P R (1968). '**Longevity and protein intake.**' *Experimental gerontology*. 3(3), pp. 231-4.

Min, et al., 2007 — Min, Kyung-Jin; Flatt, Thomas; Kulaots, Indrek; & Tatar, Marc (2007). '**Counting calories in Drosophila diet restriction.**' *Experimental gerontology*. 42(3), pp. 247-51.

Min & Tatar, 2006 — Min, Kyung-Jin & Tatar, Marc (2006). '**Restriction of amino acids extends lifespan in Drosophila melanogaster.**' *Mechanisms of ageing and development*. 127(7), pp. 643-6.

Minois, 2014 — Minois, Nadege (2014). **"Molecular basis of the 'anti-aging' effect of spermidine and other natural polyamines - a mini-review."** Gerontology. 2014;60(4):319-26. doi: 10.1159/000356748. Epub 2014 Jan 28.

Misteli, 2010 — Misteli, Tom (2010). '**Higher-order genome organization in human disease.**' *Cold Spring Harbor perspectives in biology*. 2(8), pp. a000794.

MMPT, 2016 — **'Major Mouse Testing Program.'** (2016) <http://majormouse.org/> Retrieved 2016.

Moeslinger, et al., 2006 — Moeslinger, Thomas; Friedl, Roswitha; & Spieckermann, Paul Gerhard (2006). '**Inhibition of inducible nitric oxide synthesis by azathioprine in a macrophage cell line.**' *Life sciences*. 79(4), pp. 374-81.

Mookerjee, et al., 2010 — Mookerjee, Shona A; Divakaruni, Ajit S; Jastroch, Martin; Brand, Martin D (2010). **"Mitochondrial uncoupling and lifespan."** Mech Ageing Dev. 2010 Jul-Aug;131(7-8):463-72. doi: 10.1016/j.mad.2010.03.010. Epub 2010 Apr 2.

Morgan, 2003 — Morgan, Shelly (2003). '**The Okinawa Diet: The key to longevity?**'.

Moroz, et al., 2014 — Moroz, Natalie; Carmona, Juan J; Anderson, Edward; Hart, Anne C; Sinclair, David A; & Blackwell, T Keith (2014). '**Dietary restriction involves NAD(+) -dependent mechanisms and a shift toward oxidative metabolism.**' *Aging cell*. 13(6), pp. 1075-85.

Morozova & Marra, 2008 — Morozova, Olena & Marra, Marco A (2008). '**Applications of next-generation sequencing technologies in functional genomics.**' *Genomics*. 92(5), pp. 255-64.

Morselli, et al., 2009 — Morselli, Eugenia; Galluzzi, Lorenzo; Kepp, Oliver; Criollo, Alfredo; Maiuri, Maria Chiara; Tavernarakis, Nektarios; Madeo, Frank; & Kroemer, Guido (2009). '**Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol.**' *Aging*. 1(12), pp. 961-70.es pharmacological lifespan extension by spermidine and resveratrol."** Aging (Albany NY). 2009 Dec 23;1(12):961-70.

Most, et al., 2016 — Most, Jasper; Tosti, Valeria; Redman, Leanne M; & Fontana, Luigi (2016). '**Calorie restriction in humans: An update.**' *Ageing research reviews*.

Muskhelishvili, et al., 1995 — Muskhelishvili, L; Hart, R W; Turturro, A; & James, S J (1995). '**Age-related changes in the intrinsic rate of apoptosis in livers of diet-restricted and ad libitum-fed B6C3F1 mice.**' *The American journal of pathology*. 147(1), pp. 20-4.

Nadon, et al., 2016 — Nadon, Nancy L; Strong, Randy; Miller, Richard A; & Harrison, David E (2016). '**NIA Interventions Testing Program: Investigating Putative Aging Intervention Agents in a Genetically Heterogeneous Mouse Model.**' *EBioMedicine*. .

Naidoo, et al., 2008 Naidoo, Nirinjini; Ferber, Megan; Master, Monali; Zhu, Yan; & Pack, Allan I (2008). '**Aging impairs the unfolded protein response to sleep deprivation and leads to proapoptotic signaling.**' *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 28(26), pp. 6539-48.

Naik, 2012 Naik, Gautam (2012). '**Big Calorie Cuts Don't Equal Longer Life, Study Suggests.**' *The Wallstreet Journal*.

Nair, 2005 Nair, K Sreekumaran (2005). '**Aging muscle.**' *The American journal of clinical nutrition*. 81(5), pp. 953-63.

Naito, et al., 2007 Naito, Hisao; Kamijima, Michihiro; Yamanoshita, Osamu; Nakahara, Ai; Katoh, Takahiko; Tanaka, Naoki; Aoyama, Toshifumi; Gonzalez, Frank J; & Nakajima, Tamie (2007). '**Differential effects of aging, drinking and exercise on serum cholesterol levels dependent on the PPARA-V227A polymorphism.**' *Journal of occupational health*. 49(5), pp. 353-62.

Nardai, et al., 2002 Nardai, Gabor; Csermely, Peter; Soti, Csaba (2002). **"Chaperone function and chaperone overload in the aged. A preliminary analysis."** Exp Gerontol. 2002 Oct-Nov;37(10-11):1257-62.

Natsume-Kitatani, et al., 2011 Natsume-Kitatani, Yayoi; Shiga, Motoki; & Mamitsuka, Hiroshi (2011). '**Genome-wide integration on transcription factors, histone acetylation and gene expression reveals genes co-regulated by histone modification patterns.**' *PloS one*. 6(7), pp. e22281.

Ng, et al., 2010 Ng, Sheau-Fang; Lin, Ruby C Y; Laybutt, D Ross; Barres, Romain; Owens, Julie A; & Morris, Margaret J (2010). '**Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring.**' *Nature*. 467(7318), pp. 963-6.

Nikko & Pelham, 2009 Nikko, Elina & Pelham, Hugh R B (2009). '**Arrestin-mediated endocytosis of yeast plasma membrane transporters.**' *Traffic (Copenhagen, Denmark)*. 10(12), pp. 1856-67.

Nishimura, et al., 2014 Nishimura, Mayuko; Kumsta, Caroline; Kaushik, Gaurav; Diop, Soda B; Ding, Yun; Bisharat-Kernizan, Jumana; Catan, Hannah; Cammarato, Anthony; Ross, Robert S; Engler, Adam J; Bodmer, Rolf; Hansen, Malene; Ocorr, Karen (2014). **"A dual role for integrin-linked kinase and beta1-integrin in modulating cardiac aging."** Aging Cell. 2014 Jun;13(3):431-40. doi: 10.1111/acel.12193. Epub 2014 Jan 9.

Nurkovic, et al., 2016 Nurkovic, Jasmin; Volarevic, Vladislav; Lako, Majlinda; Armstrong, Lyle; Arsenijevic, Nebojsa; & Stojkovic, Miodrag (2016). '**Aging of Stem and Progenitor Cells: Mechanisms, Impact on Therapeutic Potential, and Rejuvenation.**' *Rejuvenation research*. 19(1), pp. 3-12.

Nygaard, et al., 2013 Nygaard, Marianne; Soerensen, Mette; Flachsbart, Friederike; Mengel-From, Jonas; Tan, Qihua; Schreiber, Stefan; Nebel, Almut; Christensen, Kaare; & Christiansen, Lene (2013). '**AKT1 fails to replicate as a longevity-associated gene in Danish and German nonagenarians and centenarians.**' *European journal of human genetics : EJHG*. 21(5), pp. 574-7.

Okuyama, et al., 2010 Okuyama, Tetsuya; Inoue, Hideki; Ookuma, Sadatsugu; Satoh, Takayuki; Kano, Kei; Honjoh, Sakiko; Hisamoto, Naoki; Matsumoto, Kunihiro; Nishida, Eisuke (2010). **"The ERK-MAPK pathway regulates longevity through SKN-1 and insulin-like signaling in Caenorhabditis elegans."** J Biol Chem. 2010 Sep 24;285(39):30274-81. doi: 10.1074/jbc.M110.146274. Epub 2010 Jul 12.

Oling, et al., 2014 Oling, David; Eisele, Frederik; Kvint, Kristian; Nystrom, Thomas (2014). **"Opposing roles of Ubp3-dependent deubiquitination regulate replicative life span and heat resistance."** EMBO J. 2014 Apr 1;33(7):747-61. doi: 10.1002/embj.201386822. Epub 2014 Mar 4.

Olsen & Kaeberlein, 2017 Brady Olsen. **"Lifespan Observations Database"** <http://lifespandb.sageweb.org/>

Olthof, et al., 2000 Olthof, M R; Hollman, P C; Vree, T B; & Katan, M B (2000). '**Bioavailabilities of quercetin-3-glucoside and quercetin-4'-glucoside do not differ in humans.**' *The Journal of nutrition*. 130(5), pp. 1200-3.

Omodei & Fontana, 2011 Omodei, Daniela & Fontana, Luigi (2011). '**Calorie restriction and prevention of age-associated chronic disease.**' *FEBS letters*. 585(11), pp. 1537-42.

Onken & Driscoll, 2010 — Onken, Brian & Driscoll, Monica (2010). '**Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1.**' *PloS one*. 5(1), pp. e8758.

Ooka, et al., 1988 — Ooka, H; Segall, P E; & Timiras, P S (1988). '**Histology and survival in age-delayed low-tryptophan-fed rats.**' *Mechanisms of ageing and development*. 43(1), pp. 79-98.

Opgen-Rhein & Strimmer, 2007 — Opgen-Rhein, Rainer & Strimmer, Korbinian (2007). '**From correlation to causation networks: a simple approximate learning algorithm and its application to high-dimensional plant gene expression data.**' *BMC systems biology*. 1, pp. 37.

Orentreich, et al., 1993 — Orentreich, N; Matias, J R; DeFelice, A; & Zimmerman, J A (1993). '**Low methionine ingestion by rats extends life span.**' *The Journal of nutrition*. 123(2), pp. 269-74.

Ori, et al., 2015 — Ori, Alessandro; Toyama, Brandon H; Harris, Michael S; Bock, Thomas; Iskar, Murat; Bork, Peer; Ingolia, Nicholas T; Hetzer, Martin W; & Beck, Martin (2015). '**Integrated Transcriptome and Proteome Analyses Reveal Organ-Specific Proteome Deterioration in Old Rats.**' *Cell systems*. 1(3), pp. 224-37.

Orom, et al., 2012 — Orom, Ulf Andersson; Lim, Meng K; Savage, Jason E; Jin, Lianjin; Saleh, Anthony D; Lisanti, Michael P; & Simone, Nicole L (2012). '**MicroRNA-203 regulates caveolin-1 in breast tissue during caloric restriction.**' *Cell cycle (Georgetown, Tex.)*. 11(7), pp. 1291-5.

Orozco-Solis+Sassone-Corsi, 2014 — Orozco-Solis, Ricardo & Sassone-Corsi, Paolo (2014). '**Circadian clock: linking epigenetics to aging.**' *Current opinion in genetics & development*. 26, pp. 66-72.

Ortt & Sinha, 2006 — Ortt, Kori & Sinha, Satrajit (2006). '**Derivation of the consensus DNA-binding sequence for p63 reveals unique requirements that are distinct from p53.**' *FEBS letters*. 580(18), pp. 4544-50.

Osiewacz & Kimpel, 1999 — Osiewacz, H D & Kimpel, E (1999). '**Mitochondrial-nuclear interactions and lifespan control in fungi.**' *Experimental gerontology*. 34(8), pp. 901-9.

Ostlund, et al., 2010 — Ostlund G, Schmitt T, Forslund K, Kostler T, Messina DN, Roopra S, Frings O, & Sonnhammer EL (2010) '**InParanoid 7: new algorithms and tools for eukaryotic orthology analysis**', *Nucleic acids research*, 38(Database issue), pp.D196-203. [PMID:19892828]

Ott, et al., 2014 — Ott, Christiane; Jacobs, Kathleen; Haucke, Elisa; Navarrete Santos, Anne; Grune, Tilman; & Simm, Andreas (2014). '**Role of advanced glycation end products in cellular signaling.**' *Redox biology*. 2, pp. 411-29.

Ouyang, et al., 2011 — Ouyang, Yingshi; Song, Yan; & Lu, Bingwei (2011). '**dp53 Restrains ectopic neural stem cell formation in the Drosophila brain in a non-apoptotic mechanism involving Archipelago and cyclin E.**' *PloS one*. 6(11), pp. e28098.

Owen, et al., 2003 — Owen, Art B; Stuart, Josh; Mach, Kathy; Villeneuve, Anne M; & Kim, Stuart (2003). '**A gene recommender algorithm to identify coexpressed genes in C. elegans.**' *Genome research*. 13(8), pp. 1828-37.

Paik, et al., 2007 — Paik, Ji-Hye; Kollipara, Ramya; Chu, Gerald; Ji, Hongkai; Xiao, Yonghong; Ding, Zhihu; Miao, Lili; Tothova, Zuzana; Horner, James W; Carrasco, Daniel R; Jiang, Shan; Gilliland, D Gary; Chin, Lynda; Wong, Wing H; Castrillon, Diego H; & DePinho, Ronald A (2007). '**FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis.**' *Cell*. 128(2), pp. 309-23.

Paik, et al., 2009 — Paik, Ji-hye; Ding, Zhihu; Narurkar, Rujuta; Ramkissoon, Shakti; Muller, Florian; Kamoun, Walid S; Chae, Sung-Suk; Zheng, Hongwu; Ying, Haoqiang; Mahoney, Jed; Hiller, David; Jiang, Shan; Protopopov, Alexei; Wong, Wing H; Chin, Lynda; Ligon, Keith L; & DePinho, Ronald A (2009). '**FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis.**' *Cell stem cell*. 5(5), pp. 540-53.

Palikaras, et al., 2016 — Palikaras, Konstantinos; Mari, Meropi; Petanidou, Barbara; Pasparaki, Angela; Filippidis, George; Tavernarakis, Nektarios (2016). **"Ectopic fat deposition contributes to age-associated pathology in Caenorhabditis elegans."** J Lipid Res. 2016 Nov 24. pii: jlr.M069385.

Pamplona & Barja, 2006 — Pamplona, Reinald & Barja, Gustavo (2006). '**Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection.**' *Biochimica et biophysica acta*. 1757(5-6), pp. 496-508.

Pan, 2002 — Pan, Wei (2002). '**A comparative review of statistical methods for discovering differentially expressed genes in replicated microarray experiments.**' *Bioinformatics (Oxford, England)*. 18(4), pp. 546-54.

Pang, et al., 2004 — Pang, Kevin C H; Miller, Jonathan P; McAuley, J Devin (2004). **"Circadian rhythms in SAMP8: a longitudinal study of the effects of age and experience."** Neurobiol Aging. 2004 Jan;25(1):111-23.

Paracelsus, 1965 — Paracelsus (1965) '**Die dritte Defension wegen des Schreibens der neuen Rezepte.**' *Septem Defensiones*. 1538. Werke Bd. 2, Darmstadt 1965, p. 510

Partridge, et al., 2005 — Partridge, Linda; Piper, Matthew D W; & Mair, William (2005). '**Dietary restriction in Drosophila.**' *Mechanisms of ageing and development*. 126(9), pp. 938-50.

Pawlikowska, et al., 2009 — Pawlikowska, Ludmila; Hu, Donglei; Huntsman, Scott; Sung, Andrew; Chu, Catherine; Chen, Justin; Joyner, Alexander H; Schork, Nicholas J; Hsueh, Wen-Chi; Reiner, Alexander P; Psaty, Bruce M; Atzmon, Gil; Barzilai, Nir; Cummings, Steven R; Browner, Warren S; Kwok, Pui-Yan; & Ziv, Elad (2009). '**Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity.**' *Aging cell*. 8(4), pp. 460-72.

Pearson, 2006 — Pearson, Helen (2006). '**Cool mice live longer.**' *Nature*, doi:10.1038/news061030-11.

Pegoraro, et al., 2009 — Pegoraro, Gianluca; Kubben, Nard; Wickert, Ute; Gohler, Heike; Hoffmann, Katrin; & Misteli, Tom (2009). '**Ageing-related chromatin defects through loss of the NURD complex.**' *Nature cell biology*. 11(10), pp. 1261-7.

Penner, et al., 2016 — Penner, M R; Parrish, R R; Hoang, L T; Roth, T L; Lubin, F D; & Barnes, C A (2016). '**Age-related changes in Egr1 transcription and DNA methylation within the hippocampus.**' *Hippocampus*. 26(8), pp. 1008-20.

Pereira, et al., 2007 — Pereira, Ana Carolina; Wu, William; & Small, Scott A (2007). '**Imaging-guided microarray: isolating molecular profiles that dissociate Alzheimer's disease from normal aging.**' *Annals of the New York Academy of Sciences*. 1097, pp. 225-38.

Perez, et al., 2007 — Perez, C A; Ott, J; Mays, D J; & Pietenpol, J A (2007). '**p63 consensus DNA-binding site: identification, analysis and application into a p63MH algorithm.**' *Oncogene*. 26(52), pp. 7363-70.

Perez-Jimenez, et al., 2005 — Perez-Jimenez, F; Alvarez de Cienfuegos, G; Badimon, L; Barja, G; Battino, M; Blanco, A; Bonanome, A; Colomer, R; Corella-Piquer, D; Covas, I; Chamorro-Quiros, J; Escrich, E; Gaforio, J J; Garcia Luna, P P; Hidalgo, L; Kafatos, A; Kris-Etherton, P M; Lairon, D; Lamuela-Raventos, R; Lopez-Miranda, J; Lopez-Segura, F; Martinez-Gonzalez, M A; Mata, P; Mataix, J; Ordovas, J; Osada, J; Pacheco-Reyes, R; Perucho, M; Pineda-Priego, M; Quiles, J L; Ramirez-Tortosa, M C; Ruiz-Gutierrez, V; Sanchez-Rovira, P; Solfrizzi, V; Soriguer-Escofet, F; de la Torre-Fornell, R; Trichopoulos, A; Villalba-Montoro, J M; Villar-Ortiz, J R; & Visioli, F (2005). '**International conference on the healthy effect of virgin olive oil.**' *European journal of clinical investigation*. 35(7), pp. 421-4.

Phillip, et al., 2015 — Phillip, Jude M; Aifuwa, Ivie; Walston, Jeremy; Wirtz, Denis (2015). **"The Mechanobiology of Aging."** Annu Rev Biomed Eng. 2015;17:113-41. doi: 10.1146/annurev-bioeng-071114-040829.

Pienta, et al., 1992 — Pienta, K J; Getzenberg, R H; Coffey, D S (1992). **"Characterization of nuclear morphology and nuclear matrices in ageing human fibroblasts."** Mech Ageing Dev. 1992 Jan;62(1):13-24.

Pinkston, et al., 2006 — Pinkston, Julie M; Garigan, Delia; Hansen, Malene; & Kenyon, Cynthia (2006). '**Mutations that increase the life span of C. elegans inhibit tumor growth.**' *Science (New York, N.Y.)*. 313(5789), pp. 971-5.

Piper, 2006 — Piper, Peter W (2006). '**Long-lived yeast as a model for ageing research.**' *Yeast (Chichester, England)*. 23(3), pp. 215-26.

Pittman, 2007 — Pittman, Joyce (2007). **"Effect of aging on wound healing: current concepts."** J Wound Ostomy Continence Nurs. 2007 Jul-Aug;34(4):412-5; quiz 416-7.

Place & Cruickshank, 2010 — Place, Ned J & Cruickshank, Jenifer (2010). '**Short photoperiod initiated during adulthood sustains reproductive function in older female siberian hamsters more effectively than short photoperiod initiated before puberty.**' *Biology of reproduction*. 82(4), pp. 778-82.

Plank, et al., 2012 — Plank, Michael; Wuttke, Daniel; van Dam, Sipko; Clarke, Susan A; & de Magalhaes, Joao Pedro (2012). '**A meta-analysis of caloric restriction gene expression profiles to infer common signatures and regulatory mechanisms.**' *Molecular bioSystems*. 8(4), pp. 1339-49.

Pletcher, et al., 2002 — Pletcher, Scott D; Macdonald, Stuart J; Marguerie, Richard; Certa, Ulrich; Stearns, Stephen C; Goldstein, David B; & Partridge, Linda (2002). '**Genome-wide transcript profiles in aging and calorically restricted Drosophila melanogaster.**' *Current biology : CB*. 12(9), pp. 712-23.

Pnueli, et al., 2004 — Pnueli, Lilach; Edry, Iris; Cohen, Miriam; & Kassir, Yona (2004). '**Glucose and nitrogen regulate the switch from histone deacetylation to acetylation for expression of early meiosis-specific genes in budding yeast.**' *Molecular and cellular biology*. 24(12), pp. 5197-208.

Pokholok, et al., 2005 — Pokholok, Dmitry K; Harbison, Christopher T; Levine, Stuart; Cole, Megan; Hannett, Nancy M; Lee, Tong Ihn; Bell, George W; Walker, Kimberly; Rolfe, P Alex; Herbolsheimer, Elizabeth; Zeitlinger, Julia; Lewitter, Fran; Gifford, David K; & Young, Richard A (2005). '**Genome-wide map of nucleosome acetylation and methylation in yeast.**' *Cell*. 122(4), pp. 517-27.

Pollina & Brunet, 2011 — Pollina, E A & Brunet, A (2011). '**Epigenetic regulation of aging stem cells.**' *Oncogene*. 30(28), pp. 3105-26.

Powers, et al., 2009 — Powers, Evan T; Morimoto, Richard I; Dillin, Andrew; Kelly, Jeffery W; & Balch, William E (2009). '**Biological and chemical approaches to diseases of proteostasis deficiency.**' *Annual review of biochemistry*. 78, pp. 959-91.

Pozzi, et al., 2009 — Pozzi, Silvia; Boergesen, Michael; Sinha, Satrajit; Mandrup, Susanne; & Mantovani, Roberto (2009). '**Peroxisome proliferator-activated receptor-alpha is a functional target of p63 in adult human keratinocytes.**' *The Journal of investigative dermatology*. 129(10), pp. 2376-85.

Prat & Roberge, 1960 — Prat, H & Roberge, L (1960). '**[Variations of thermogenesis in function of aging in the mouse].**' *Revue canadienne de biologie*. 19, pp. 80-93.

Price, et al., 2012 — Price, Nathan L; Gomes, Ana P; Ling, Alvin J Y; Duarte, Filipe V; Martin-Montalvo, Alejandro; North, Brian J; Agarwal, Beamon; Ye, Lan; Ramadori, Giorgio; Teodoro, Joao S; Hubbard, Basil P; Varela, Ana T; Davis, James G; Varamini, Behzad; Hafner, Angela; Moaddel, Ruin; Rolo, Anabela P; Coppari, Roberto; Palmeira, Carlos M; de Cabo, Rafael; Baur, Joseph A; & Sinclair, David A (2012). '**SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function.**' *Cell metabolism*. 15(5), pp. 675-90.

Promislow, 2004 — Promislow, Daniel E L (2004). **"Protein networks, pleiotropy and the evolution of senescence."** Proc Biol Sci. 2004 Jun 22;271(1545):1225-34.

Quick, 2015 — Darren Quick (2015) '**Senolytics: A new class of drugs with the potential to slow the aging process.**', Available from http://www.gizmag.com/senolytics-drug-slow-aging/36472(). [Retrieved March 10, 2015]

Raes, 1991 — Raes, M (1991). **"Involvement of microtubules in modifications associated with cellular aging."** Mutat Res. 1991 Mar-Nov;256(2-6):149-68.

Raimondo, et al., 2016 — Raimondo, Lucia; D'Amato, Valentina; Servetto, Alberto; Rosa, Roberta; Marciano, Roberta; Formisano, Luigi; Di Mauro, Concetta; Orsini, Roberta Clara; Cascetta, Priscilla; Ciciola, Paola; De Maio, Ana Paula; Di Renzo, Maria Flavia; Cosconati, Sandro; Bruno, Agostino; Randazzo, Antonio; Napolitano, Filomena; Montuori, Nunzia; Veneziani, Bianca Maria; De Placido, Sabino; & Bianco, Roberto (2016). '**Everolimus induces Met inactivation by disrupting the FKBP12/Met complex.**' *Oncotarget*. 7(26), pp. 40073-40084.

Rakyan, et al., 2010 Rakyan, Vardhman K; Down, Thomas A; Maslau, Siarhei; Andrew, Toby; Yang, Tsun-Po; Beyan, Huriya; Whittaker, Pamela; McCann, Owen T; Finer, Sarah; Valdes, Ana M; Leslie, R David; Deloukas, Panogiotis; & Spector, Timothy D (2010). '**Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains.**' *Genome research*. 20(4), pp. 434-9.

Rattan, 2007 Rattan, Suresh I S (2007). '**The science of healthy aging: genes, milieu, and chance.**' *Annals of the New York Academy of Sciences*. 1114, pp. 1-10.

Ravasi, et al., 2010 Ravasi, Timothy; Suzuki, Harukazu; Cannistraci, Carlo Vittorio; Katayama, Shintaro; Bajic, Vladimir B; Tan, Kai; Akalin, Altuna; Schmeier, Sebastian; Kanamori-Katayama, Mutsumi; Bertin, Nicolas; Carninci, Piero; Daub, Carsten O; Forrest, Alistair R R; Gough, Julian; Grimmond, Sean; Han, Jung-Hoon; Hashimoto, Takehiro; Hide, Winston; Hofmann, Oliver; Kamburov, Atanas; Kaur, Mandeep; Kawaji, Hideya; Kubosaki, Atsutaka; Lassmann, Timo; van Nimwegen, Erik; MacPherson, Cameron Ross; Ogawa, Chihiro; Radovanovic, Aleksandar; Schwartz, Ariel; Teasdale, Rohan D; Tegner, Jesper; Lenhard, Boris; Teichmann, Sarah A; Arakawa, Takahiro; Ninomiya, Noriko; Murakami, Kayoko; Tagami, Michihira; Fukuda, Shiro; Imamura, Kengo; Kai, Chikatoshi; Ishihara, Ryoko; Kitazume, Yayoi; Kawai, Jun; Hume, David A; Ideker, Trey; & Hayashizaki, Yoshihide (2010). '**An atlas of combinatorial transcriptional regulation in mouse and man.**' *Cell*. 140(5), pp. 744-52.

Razick, et al., 2008 Razick, Sabry; Magklaras, George; & Donaldson, Ian M (2008). '**iRefIndex: a consolidated protein interaction database with provenance.**' *BMC bioinformatics*. 9, pp. 405.

Reinke, et al., 2006 Reinke, Aaron; Chen, Jenny C-Y; Aronova, Sofia; & Powers, Ted (2006). '**Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p.**' *The Journal of biological chemistry*. 281(42), pp. 31616-26.

Reis-Rodrigues, et al., 2012 Reis-Rodrigues, Pedro; Czerwieniec, Gregg; Peters, Theodore W; Evani, Uday S; Alavez, Silvestre; Gaman, Emily A; Vantipalli, Maithili; Mooney, Sean D; Gibson, Bradford W; Lithgow, Gordon J; & Hughes, Robert E (2012). '**Proteomic analysis of age-dependent changes in protein solubility identifies genes that modulate lifespan.**' *Aging cell*. 11(1), pp. 120-7.

Renault, et al., 2009 Renault, Valerie M; Rafalski, Victoria A; Morgan, Alex A; Salih, Dervis A M; Brett, Jamie O; Webb, Ashley E; Villeda, Saul A; Thekkat, Pramod U; Guillerey, Camille; Denko, Nicholas C; Palmer, Theo D; Butte, Atul J; & Brunet, Anne (2009). '**FoxO3 regulates neural stem cell homeostasis.**' *Cell stem cell*. 5(5), pp. 527-39.

Renaville, et al., 2002 Renaville, R; Hammadi, M; & Portetelle, D (2002). '**Role of the somatotropic axis in the mammalian metabolism.**' *Domestic animal endocrinology*. 23(1-2), pp. 351-60.

Ressler, et al., 2006 Ressler, Sigrun; Bartkova, Jirina; Niederegger, Harald; Bartek, Jiri; Scharffetter-Kochanek, Karin; Jansen-Durr, Pidder; & Wlaschek, Meinhard (2006). '**p16INK4A is a robust in vivo biomarker of cellular aging in human skin.**' *Aging cell*. 5(5), pp. 379-89.

Rhie, et al., 2013 Rhie, Byung-Ho; Song, Young-Ha; Ryu, Hong-Yeoul; Ahn, Seong Hoon (2013). **"Cellular aging is associated with increased ubiquitylation of histone H2B in yeast telomeric heterochromatin."** Biochem Biophys Res Commun. 2013 Oct 4;439(4):570-5. doi: 10.1016/j.bbrc.2013.09.017. Epub 2013 Sep 8.

Richie, et al., 1994 Richie, J P Jr; Leutzinger, Y; Parthasarathy, S; Malloy, V; Orentreich, N; & Zimmerman, J A (1994). '**Methionine restriction increases blood glutathione and longevity in F344 rats.**' *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 8(15), pp. 1302-7.

Rochon, et al., 2011 Rochon, James; Bales, Connie W; Ravussin, Eric; Redman, Leanne M; Holloszy, John O; Racette, Susan B; Roberts, Susan B; Das, Sai Krupa; Romashkan, Sergei; Galan, Katherine M; Hadley, Evan C; & Kraus, William E (2011). '**Design and conduct of the CALERIE study: comprehensive assessment of the long-term effects of reducing intake of energy.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 66(1), pp. 97-108.

Rodier & Campisi, 2011 Rodier, Francis; Campisi, Judith (2011). **"Four faces of cellular senescence."** J Cell Biol. 2011 Feb 21;192(4):547-56. doi: 10.1083/jcb.201009094. Epub 2011 Feb 14.

Rodrigue, et al., 2012 Rodrigue, K M; Kennedy, K M; Devous, M D Sr; Rieck, J R; Hebrank, A C; Diaz-Arrastia, R; Mathews, D; Park, D C (2012). **"beta-Amyloid burden in healthy aging: regional distribution and cognitive consequences."** Neurology. 2012 Feb 7;78(6):387-95. doi: 10.1212/WNL.0b013e318245d295. Epub 2012 Feb 1.

Rodriguez, et al., 2010 Rodriguez, Karl A; Gaczynska, Maria; & Osmulski, Pawel A (2010). '**Molecular mechanisms of proteasome plasticity in aging.**' *Mechanisms of ageing and development*. 131(2), pp. 144-55.

Rogina & Helfand, 1995 Rogina, B & Helfand, S L (1995). '**Regulation of gene expression is linked to life span in adult Drosophila.**' *Genetics*. 141(3), pp. 1043-8.

Rogina & Helfand, 2004 Rogina, Blanka & Helfand, Stephen L (2004). '**Sir2 mediates longevity in the fly through a pathway related to calorie restriction.**' *Proceedings of the National Academy of Sciences of the United States of America*. 101(45), pp. 15998-6003.

Rohani, et al., 2014 Rohani, Leili; Johnson, Adiv A; Arnold, Antje; & Stolzing, Alexandra (2014). '**The aging signature: a hallmark of induced pluripotent stem cells?**' *Aging cell*. 13(1), pp. 2-7.

Ross, 1961 Ross, M H (1961). '**Length of life and nutrition in the rat.**' *The Journal of nutrition*. 75, pp. 197-210.

Ross & Bras, 1975 Ross, M H & Bras, G (1975). '**Food preference and length of life.**' *Science (New York, N.Y.)*. 190(4210), pp. 165-7.

Roth, et al., 2014 Roth, Daniela Martino; Hutt, Darren M; Tong, Jiansong; Bouchecareilh, Marion; Wang, Ning; Seeley, Theo; Dekkers, Johanna F; Beekman, Jeffrey M; Garza, Dan; Drew, Lawrence; Masliah, Eliezer; Morimoto, Richard I; & Balch, William E (2014). '**Modulation of the maladaptive stress response to manage diseases of protein folding.**' *PLoS biology*. 12(11), pp. e1001998.

Rothstein, 1982 Rothstein, M. (1982) '**Biochemical Approaches to Aging.**' Academic Press, New York.

Rothwell & Stock, 1983 Rothwell, N J & Stock, M J (1983). '**Effects of age on diet-induced thermogenesis and brown adipose tissue metabolism in the rat.**' *International journal of obesity*. 7(6), pp. 583-9.

Roy, et al., 2002 Roy, Arun K; Oh, Thomas; Rivera, Omar; Mubiru, James; Song, Chung S; & Chatterjee, Bandana (2002). '**Impacts of transcriptional regulation on aging and senescence.**' *Ageing research reviews*. 1(3), pp. 367-80.

Rubinsztein, et al., 2011 Rubinsztein, David C; Marino, Guillermo; & Kroemer, Guido (2011). '**Autophagy and aging.**' *Cell*. 146(5), pp. 682-95.

Ruepp, et al., 2010 Ruepp, Andreas; Waegele, Brigitte; Lechner, Martin; Brauner, Barbara; Dunger-Kaltenbach, Irmtraud; Fobo, Gisela; Frishman, Goar; Montrone, Corinna; & Mewes, H-Werner (2010). '**CORUM: the comprehensive resource of mammalian protein complexes--2009.**' *Nucleic acids research*. 38(Database issue), pp. D497-501.

Rundlett, et al., 1998 Rundlett, S E; Carmen, A A; Suka, N; Turner, B M; & Grunstein, M (1998). '**Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3.**' *Nature*. 392(6678), pp. 831-5.

Ryu, et al., 2007 Ryu, S J; Oh, Y S; Park, S C (2007). **"Failure of stress-induced downregulation of Bcl-2 contributes to apoptosis resistance in senescent human diploid fibroblasts."** Cell Death Differ. 2007 May;14(5):1020-8. Epub 2007 Feb 9.

Saeed & Deane, 2006 Saeed, Ramazan & Deane, Charlotte M (2006). '**Protein protein interactions, evolutionary rate, abundance and age.**' *BMC bioinformatics*. 7, pp. 128.

Salminen, et al., 2011 Salminen, Antero; Ojala, Johanna; Kaarniranta, Kai (2011). **"Apoptosis and aging: increased resistance to apoptosis enhances the aging process."** Cell Mol Life Sci. 2011 Mar;68(6):1021-31. doi: 10.1007/s00018-010-0597-y. Epub 2010 Nov 30.

Salminen, et al., 2012 Salminen, Antero; Kaarniranta, Kai; & Kauppinen, Anu (2012). '**Inflammaging: disturbed interplay between autophagy and inflammasomes.**' *Aging*. 4(3), pp. 166-75.

Salvador, et al., 2016 Salvador, Laura; Singaravelu, Gunasekaran; Harley, Calvin B; Flom, Peter; Suram, Anitha; & Raffaele, Joseph M (2016). '**A Natural Product Telomerase Activator Lengthens Telomeres in Humans: A Randomized, Double Blind, and Placebo Controlled Study.**' *Rejuvenation research*.

Samson, et al., 1979 Samson, F; Donoso, J A; Heller-Bettinger, I; Watson, D; & Himes, R H (1979). '**Nocodazole action on tubulin assembly, axonal ultrastructure and fast axoplasmic transport.**' *The Journal of pharmacology and experimental therapeutics*. 208(3), pp. 411-7.

Sanchez-Roman, et al., 2011 Sanchez-Roman, Ines; Gomez, Alexia; Gomez, Jose; Suarez, Henar; Sanchez, Carlota; Naudi, Alba; Ayala, Victoria; Portero-Otin, Manuel; Lopez-Torres, Monica; Pamplona, Reinald; & Barja, Gustavo (2011). '**Forty percent methionine restriction lowers DNA methylation, complex I ROS generation, and oxidative damage to mtDNA and mitochondrial proteins in rat heart.**' *Journal of bioenergetics and biomembranes*. 43(6), pp. 699-708.

Sanderson, et al., 2004 Sanderson, L; Taylor, G W; Aboagye, E O; Alao, J P; Latigo, J R; Coombes, R C; & Vigushin, D M (2004). '**Plasma pharmacokinetics and metabolism of the histone deacetylase inhibitor trichostatin a after intraperitoneal administration to mice.**' *Drug metabolism and disposition: the biological fate of chemicals*. 32(10), pp. 1132-8.

Sangai, et al., 2012 Sangai, Takafumi; Akcakanat, Argun; Chen, Huiqin; Tarco, Emily; Wu, Yun; Do, Kim-Anh; Miller, Todd W; Arteaga, Carlos L; Mills, Gordon B; Gonzalez-Angulo, Ana Maria; & Meric-Bernstam, Funda (2012). '**Biomarkers of response to Akt inhibitor MK-2206 in breast cancer.**' *Clinical cancer research : an official journal of the American Association for Cancer Research*. 18(20), pp. 5816-28.

Sato, et al., 2006 Sato, Eriko; Yano, Ikuko; Jiko, Mari; Takahashi, Kazushige; Motohashi, Hideyuki; Masuda, Satohiro; Katsura, Toshiya; Nishiyama, Hiroyuki; Segawa, Takehiko; Ito, Noriyuki; Kamoto, Toshiyuki; Ogawa, Osamu; & Inui, Ken-ichi (2006). '**Evaluation of Calvert's formula for dosage adjustment of carboplatin in Japanese patients with hormone refractory prostate cancer.**' *Biological & pharmaceutical bulletin*. 29(7), pp. 1441-4.

Sbisa, et al., 2007 Sbisa, Elisabetta; Catalano, Domenico; Grillo, Giorgio; Licciulli, Flavio; Turi, Antonio; Liuni, Sabino; Pesole, Graziano; De Grassi, Anna; Caratozzolo, Mariano Francesco; D'Erchia, Anna Maria; Navarro, Beatriz; Tullo, Apollonia; Saccone, Cecilia; & Gisel, Andreas (2007). '**p53FamTaG: a database resource of human p53, p63 and p73 direct target genes combining in silico prediction and microarray data.**' *BMC bioinformatics*. 8 Suppl 1, pp. S20.

Scarpace, et al., 1994 Scarpace, P J; Yenice, S; Tumer, N (1994). **"Influence of exercise training and age on uncoupling protein mRNA expression in brown adipose tissue."** Pharmacol Biochem Behav. 1994 Dec;49(4):1057-9.

Schafer, et al., 2015 Schafer, Marissa J; Dolgalev, Igor; Alldred, Melissa J; Heguy, Adriana; & Ginsberg, Stephen D (2015). '**Calorie Restriction Suppresses Age-Dependent Hippocampal Transcriptional Signatures.**' *PloS one*. 10(7), pp. e0133923.

Schatz, et al., 2010 Schatz, M., Langmead, B. & Salzberg, S. Nat. Biotechnol. 28, 691–693 (2010). 8. 1000 Genomes Project data available on Amazon

Schauble, et al., 2012 Schauble, Sascha; Klement, Karolin; Marthandan, Shiva; Munch, Sandra; Heiland, Ines; Schuster, Stefan; Hemmerich, Peter; Diekmann, Stephan (2012). **"Quantitative model of cell cycle arrest and cellular senescence in primary human fibroblasts."** PLoS One. 2012;7(8):e42150. doi: 10.1371/journal.pone.0042150. Epub 2012 Aug 7.

Schneider, et al., 2017 Schneider, Augusto; Dhahbi, Joseph M; Atamna, Hani; Clark, Josef P; Colman, Ricki J; & Anderson, Rozalyn M (2017). '**Caloric restriction impacts plasma microRNAs in rhesus monkeys.**' *Aging cell*.

Schumacher, et al., 2008 Schumacher, Bjorn; van der Pluijm, Ingrid; Moorhouse, Michael J; Kosteas, Theodore; Robinson, Andria Rasile; Suh, Yousin; Breit, Timo M; van Steeg, Harry; Niedernhofer, Laura J; van Ijcken, Wilfred; Bartke, Andrzej; Spindler, Stephen R; Hoeijmakers, Jan H J; van der Horst, Gijsbertus T J; & Garinis, George A (2008). '**Delayed and accelerated aging share common longevity assurance mechanisms.**' *PLoS genetics*. 4(8), pp. e1000161.

Schuster, et al., 2010 Schuster, Eugene; McElwee, Joshua J; Tullet, Jennifer M A; Doonan, Ryan; Matthijssens, Filip; Reece-Hoyes, John S; Hope, Ian A; Vanfleteren, Jacques R; Thornton, Janet M; & Gems, David (2010). '**DamID in C. elegans reveals longevity-associated targets of DAF-16/FoxO.**' *Molecular systems biology*. 6, pp. 399.

Schwartz, et al., 2006 Schwartz, Yuri B; Kahn, Tatyana G; Nix, David A; Li, Xiao-Yong; Bourgon, Richard; Biggin, Mark; & Pirrotta, Vincenzo (2006). '**Genome-wide analysis of Polycomb targets in Drosophila melanogaster.**' *Nature genetics*. 38(6), pp. 700-5.

Sedivy, et al., 2008 Sedivy, John M; Banumathy, Gowrishankar; & Adams, Peter D (2008). '**Aging by epigenetics--a consequence of chromatin damage?**' *Experimental cell research*. 314(9), pp. 1909-17.

Selman, et al., 2006a Selman, Colin; Kerrison, Nicola D; Cooray, Anisha; Piper, Matthew D W; Lingard, Steven J; Barton, Richard H; Schuster, Eugene F; Blanc, Eric; Gems, David; Nicholson, Jeremy K; Thornton, Janet M; Partridge, Linda; & Withers, Dominic J (2006). '**Coordinated multitissue transcriptional and plasma metabonomic profiles following acute caloric restriction in mice.**' *Physiological genomics*. 27(3), pp. 187-200.

Selman, et al., 2009 Selman, Colin; Tullet, Jennifer M A; Wieser, Daniela; Irvine, Elaine; Lingard, Steven J; Choudhury, Agharul I; Claret, Marc; Al-Qassab, Hind; Carmignac, Danielle; Ramadani, Faruk; Woods, Angela; Robinson, Iain C A; Schuster, Eugene; Batterham, Rachel L; Kozma, Sara C; Thomas, George; Carling, David; Okkenhaug, Klaus; Thornton, Janet M; Partridge, Linda; Gems, David; & Withers, Dominic J (2009). '**Ribosomal protein S6 kinase 1 signaling regulates mammalian life span.**' *Science (New York, N.Y.)*. 326(5949), pp. 140-4.

Senoo, et al., 2007 Senoo, Makoto; Pinto, Filipa; Crum, Christopher P; & McKeon, Frank (2007). '**p63 Is essential for the proliferative potential of stem cells in stratified epithelia.**' *Cell*. 129(3), pp. 523-36.

Seong, et al., 2011 Seong, Ki-Hyeon; Li, Dong; Shimizu, Hideyuki; Nakamura, Ryoichi; & Ishii, Shunsuke (2011). '**Inheritance of stress-induced, ATF-2-dependent epigenetic change.**' *Cell*. 145(7), pp. 1049-61.

Seroude, 2002 Seroude, Laurent (2002). '**Differential gene expression and aging.**' *TheScientificWorldJournal*. 2, pp. 618-31.

Seroude, et al., 2002 Seroude, Laurent; Brummel, Ted; Kapahi, Pankaj; & Benzer, Seymour (2002). '**Spatio-temporal analysis of gene expression during aging in Drosophila melanogaster.**' *Aging cell*. 1(1), pp. 47-56.

Shanley & Kirkwood, 2000 Shanley, D P & Kirkwood, T B (2000). '**Calorie restriction and aging: a life-history analysis.**' *Evolution; international journal of organic evolution*. 54(3), pp. 740-50.

Sharma, et al., 2011 Sharma, Praveen Kumar; Mittal, Nitish; Deswal, Sumit; & Roy, Nilanjan (2011). '**Calorie restriction up-regulates iron and copper transport genes in Saccharomyces cerevisiae.**' *Molecular bioSystems*. 7(2), pp. 394-402.

Shaw, et al., 2010 Shaw, Albert C; Joshi, Samit; Greenwood, Hannah; Panda, Alexander; & Lord, Janet M (2010). '**Aging of the innate immune system.**' *Current opinion in immunology*. 22(4), pp. 507-13.

Shen, et al., 2003 Shen, Huo; Kannari, Kazuya; Yamato, Hiroshi; Arai, Akira; & Matsunaga, Muneo (2003). '**Effects of benserazide on L-DOPA-derived extracellular dopamine levels and aromatic L-amino acid decarboxylase activity in the striatum of 6-hydroxydopamine-lesioned rats.**' *The Tohoku journal of experimental medicine*. 199(3), pp. 149-59.

Shih & Yen, 2007 Shih, Ping-Hsiao; Yen, Gow-Chin (2007). **"Differential expressions of antioxidant status in aging rats: the role of transcriptional factor Nrf2 and MAPK signaling pathway."** Biogerontology. 2007 Apr;8(2):71-80. Epub 2006 Jul 19.

Shimokawa, et al., 1996 Shimokawa, I; Higami, Y; Yu, B P; Masoro, E J; & Ikeda, T (1996). '**Influence of dietary components on occurrence of and mortality due to neoplasms in male F344 rats.**' *Aging (Milan, Italy)*. 8(4), pp. 254-62.

Shmookler Reis, et al., 2009 Shmookler Reis, Robert J; Bharill, Puneet; Tazearslan, Cagdas; & Ayyadevara, Srinivas (2009). '**Extreme-longevity mutations orchestrate silencing of multiple signaling pathways.**' *Biochimica et biophysica acta*. 1790(10), pp. 1075-83.

Shmookler Reis, et al., 2011 Shmookler Reis, Robert J; Xu, Lulu; Lee, Hoonyong; Chae, Minho; Thaden, John J; Bharill, Puneet; Tazearslan, Cagdas; Siegel, Eric; Alla, Ramani; Zimniak, Piotr; & Ayyadevara, Srinivas (2011). '**Modulation of lipid biosynthesis contributes to stress resistance and longevity of C. elegans mutants.**' *Aging*. 3(2), pp. 125-47.

Shmookler Reis, et al., 2012 Shmookler Reis, Robert J; Ayyadevara, Srinivas; Crow, W Alex; Lee, Taewon; & Delongchamp, Robert R (2012). '**Gene categories differentially expressed in C. elegans age-1 mutants of extraordinary longevity: new insights from novel data-mining procedures.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 67(4), pp. 366-75.

Shruthi, et al., 2016 Shruthi, Karnam; Reddy, S Sreenivasa; Reddy, P Yadagiri; Shivalingam, Potula; Harishankar, Nemani; & Reddy, G Bhanuprakash (2016). '**Amelioration of neuronal cell death in a spontaneous obese rat model by dietary restriction through modulation of ubiquitin proteasome system.**' *The Journal of nutritional biochemistry*. 33, pp. 73-81.

Sidler, et al., 2013 Sidler, Corinne; Woycicki, Rafal; Ilnytskyy, Yaroslav; Metz, Gerlinde; Kovalchuk, Igor; Kovalchuk, Olga (2013). **"Immunosenescence is associated with altered gene expression and epigenetic regulation in primary and secondary immune organs."** Front Genet. 2013 Oct 18;4:211. doi: 10.3389/fgene.2013.00211. eCollection 2013.

Singh, et al., 2015 Singh, Rumani; Manchanda, Shaffi; Kaur, Taranjeet; Kumar, Sushil; Lakhanpal, Dinesh; Lakhman, Sukhwinder S; & Kaur, Gurcharan (2015). '**Middle age onset short-term intermittent fasting dietary restriction prevents brain function impairments in male Wistar rats.**' *Biogerontology*. 16(6), pp. 775-88.

Smeal, et al., 1996 Smeal, T; Claus, J; Kennedy, B; Cole, F; & Guarente, L (1996). '**Loss of transcriptional silencing causes sterility in old mother cells of S. cerevisiae.**' *Cell*. 84(4), pp. 633-42.

Smith, 2002 Smith, Richard (2002). '**In search of "non-disease".**' *BMJ (Clinical research ed.)*. 324(7342), pp. 883-5. Available from: http://www.bmj.com/content/324/7342/883.1() [PMID:11950739]

Smith, et al., 2007 Smith, Erin M; Hoi, Jia Tse; Eissenberg, Joel C; Shoemaker, James D; Neckameyer, Wendi S; Ilvarsonn, Anne M; Harshman, Lawrence G; Schlegel, Vicki L; & Zempleni, Janos (2007). '**Feeding Drosophila a biotin-deficient diet for multiple generations increases stress resistance and lifespan and alters gene expression and histone biotinylation patterns.**' *The Journal of nutrition*. 137(9), pp. 2006-12.

Smith, et al., 2008 Smith, Erica D; Tsuchiya, Mitsuhiro; Fox, Lindsay A; Dang, Nick; Hu, Di; Kerr, Emily O; Johnston, Elijah D; Tchao, Bie N; Pak, Diana N; Welton, K Linnea; Promislow, Daniel E L; Thomas, James H; Kaeberlein, Matt; Kennedy, Brian K (2008). **"Quantitative evidence for conserved longevity pathways between divergent eukaryotic species."** Genome Res. 2008 Apr;18(4):564-70. doi: 10.1101/gr.074724.107. Epub 2008 Mar 13.

Smith, et al., 2011 Smith, Jennifer J; Miller, Leslie R; Kreisberg, Richard; Vazquez, Laura; Wan, Yakun; & Aitchison, John D (2011). '**Environment-responsive transcription factors bind subtelomeric elements and regulate gene silencing.**' *Molecular systems biology*. 7, pp. 455.

Smith-Vikos, et al., 2014 Smith-Vikos, Thalyana; de Lencastre, Alexandre; Inukai, Sachi; Shlomchik, Mariel; Holtrup, Brandon; & Slack, Frank J (2014). '**MicroRNAs mediate dietary-restriction-induced longevity through PHA-4/FOXA and SKN-1/Nrf transcription factors.**' *Current biology : CB*. 24(19), pp. 2238-46.

Smith & Waterman, 1981 Smith, T F & Waterman, M S (1981). '**Identification of common molecular subsequences.**' *Journal of molecular biology*. 147(1), pp. 195-7.

Snyder-Mackler, et al., 2014 Snyder-Mackler, Noah; Somel, Mehmet; & Tung, Jenny (2014). '**Shared signatures of social stress and aging in peripheral blood mononuclear cell gene expression profiles.**' *Aging cell*. 13(5), pp. 954-7.

Soda, et al., 2009 Soda, Kuniyasu; Dobashi, Yoh; Kano, Yoshihiko; Tsujinaka, Shingo; & Konishi, Fumio (2009). '**Polyamine-rich food decreases age-associated pathology and mortality in aged mice.**' *Experimental gerontology*. 44(11), pp. 727-32.

Soerensen, et al., 2012 Soerensen M, Dato S, Tan Q, Thinggaard M, Kleindorp R, Beekman M, Jacobsen R, Suchiman HE, de Craen AJ, Westendorp RG, Schreiber S, Stevnsner T, Bohr VA, Slagboom PE, Nebel A, Vaupel JW, Christensen K, McGue M, Christiansen L (2012) **Human longevity and variation in GH/IGF-1/insulin signaling, DNA damage signaling and repair and pro/antioxidant pathway genes: cross sectional and longitudinal studies.**. *Experimental gerontology*, 47(5), pp.379-87.

Sohal & Weindruch, 1996 Sohal, R S & Weindruch, R (1996). '**Oxidative stress, caloric restriction, and aging.**' *Science (New York, N.Y.)*. 273(5271), pp. 59-63.

Somel, et al., 2010 Somel, Mehmet; Guo, Song; Fu, Ning; Yan, Zheng; Hu, Hai Yang; Xu, Ying; Yuan, Yuan; Ning, Zhibin; Hu, Yuhui; Menzel, Corinna; Hu, Hao; Lachmann, Michael; Zeng, Rong; Chen, Wei; & Khaitovich, Philipp (2010). '**MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain.**' *Genome research*. 20(9), pp. 1207-18.

Sood, et al., 2015 Sood, Sanjana; Gallagher, Iain J; Lunnon, Katie; Rullman, Eric; Keohane, Aoife; Crossland, Hannah; Phillips, Bethan E; Cederholm, Tommy; Jensen, Thomas; van Loon, Luc J C; Lannfelt, Lars; Kraus, William E; Atherton, Philip J; Howard, Robert; Gustafsson, Thomas; Hodges, Angela; & Timmons, James A (2015). '**A novel multi-tissue RNA diagnostic of healthy ageing relates to cognitive health status.**' *Genome biology*. 16, pp. 185.

Soucy, et al., 2006 Soucy, Nicole V; Parkinson, Horace D; Sochaski, Mark A; & Borghoff, Susan J (2006). '**Kinetics of genistein and its conjugated metabolites in pregnant Sprague-Dawley rats following single and repeated genistein administration.**' *Toxicological sciences : an official journal of the Society of Toxicology*. 90(1), pp. 230-40.

Southworth, et al., 2009 Southworth, Lucinda K; Owen, Art B; & Kim, Stuart K (2009). '**Aging mice show a decreasing correlation of gene expression within genetic modules.**' *PLoS genetics*. 5(12), pp. e1000776.

Spindler, 2010 Spindler, Stephen R (2010). '**Caloric restriction: from soup to nuts.**' *Ageing research reviews*. 9(3), pp. 324-53.

Stark, et al., 2010 Stark, Chris; Su, Ting-Cheng; Breitkreutz, Ashton; Lourenco, Pedro; Dahabieh, Matthew; Breitkreutz, Bobby-Joe; Tyers, Mike; & Sadowski, Ivan (2010). '**PhosphoGRID: a database of experimentally verified in vivo protein phosphorylation sites from the budding yeast Saccharomyces cerevisiae.**' *Database : the journal of biological databases and curation*. 2010, pp. bap026.

Stauch, et al., 2015 Stauch, Kelly L; Purnell, Phillip R; Villeneuve, Lance M; Fox, Howard S (2015). **"Data for mitochondrial proteomic alterations in the aging mouse brain."** Data Brief. 2015 May 21;4:127-9. doi: 10.1016/j.dib.2015.05.004. eCollection 2015 Sep.

Stavrou, 2011 Stavrou, Emmanouil (2011) **"Regulation of FOXO transcription factors by gonadotropin-releasing hormone."** <http://www.era.lib.ed.ac.uk/handle/1842/5686>

Steffen, et al., 2008 — Steffen, Kristan K; MacKay, Vivian L; Kerr, Emily O; Tsuchiya, Mitsuhiro; Hu, Di; Fox, Lindsay A; Dang, Nick; Johnston, Elijah D; Oakes, Jonathan A; Tchao, Bie N; Pak, Diana N; Fields, Stanley; Kennedy, Brian K; & Kaeberlein, Matt (2008). '**Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4.**' *Cell*. 133(2), pp. 292-302.

Stein, 2004 — Stein, Lincoln D (2004). '**Using the Reactome database.**' *Current protocols in bioinformatics*. Chapter 8, pp. Unit 8.7.

Stein, et al., 2012 — Stein, Phyllis K; Soare, Andreea; Meyer, Timothy E; Cangemi, Roberto; Holloszy, John O; & Fontana, Luigi (2012). '**Caloric restriction may reverse age-related autonomic decline in humans.**' *Aging cell*. 11(4), pp. 644-50.

Steinkraus, et al., 2008 — Steinkraus, K A; Kaeberlein, M; & Kennedy, B K (2008). '**Replicative aging in yeast: the means to the end.**' *Annual review of cell and developmental biology*. 24, pp. 29-54.

Steinkraus, et al., 2008b — Steinkraus, Katherine A; Smith, Erica D; Davis, Christina; Carr, Daniel; Pendergrass, William R; Sutphin, George L; Kennedy, Brian K; & Kaeberlein, Matt (2008). '**Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in Caenorhabditis elegans.**' *Aging cell*. 7(3), pp. 394-404.

Stoeva, 1989 — Stoeva, I (1989). '**[Gonadotropic hormones and the female sex hormones--estradiol and progesterone].**' *Akusherstvo i ginekologiia*. 28(3), pp. 70-4.

Stoltzner, 1977 — Stoltzner, G (1977). '**Effects of life-long dietary protein restriction on mortality, growth, organ weights, blood counts, liver aldolase and kidney catalase in Balb/C mice.**' *Growth*. 41(4), pp. 337-48.

Strehler, 1982 — Strehler, B. (1982) '**Time, Cells and Aging.**' *Academic Press, New York*.

Streit, et al., 1996 — Streit, F; Christians, U; Schiebel, H M; Meyer, A; & Sewing, K F (1996). '**Structural identification of three metabolites and a degradation product of the macrolide immunosuppressant sirolimus (rapamycin) by electrospray-MS/MS after incubation with human liver microsomes.**' *Drug metabolism and disposition: the biological fate of chemicals*. 24(11), pp. 1272-8.

Stroustrup, et al., 2013 — Stroustrup N1, Ulmschneider BE, Nash ZM, López-Moyado IF, Apfeld J, Fontana W. '*The Caenorhabditis elegans Lifespan Machine*' Nat Methods. 2013 Jul;10(7):665-70. doi: 10.1038/nmeth.2475. Epub 2013 May 12.

Sturgill, et al., 2008 — Sturgill, Thomas W; Cohen, Adiel; Diefenbacher, Melanie; Trautwein, Mark; Martin, Dietmar E; & Hall, Michael N (2008). '**TOR1 and TOR2 have distinct locations in live cells.**' *Eukaryotic cell*. 7(10), pp. 1819-30.

Subramanian, et al., 2017 — Subramanian, Aravind; Narayan, Rajiv; Corsello, Steven M; Peck, David D; Natoli, Ted E; Lu, Xiaodong; Gould, Joshua; Davis, John F; Tubelli, Andrew A; Asiedu, Jacob K; Lahr, David L; Hirschman, Jodi E; Liu, Zihan; Donahue, Melanie; Julian, Bina; Khan, Mariya; Wadden, David; Smith, Ian C; Lam, Daniel; Liberzon, Arthur; Toder, Courtney; Bagul, Mukta; Orzechowski, Marek; Enache, Oana M; Piccioni, Federica; Johnson, Sarah A; Lyons, Nicholas J; Berger, Alice H; Shamji, Alykhan F; Brooks, Angela N; Vrcic, Anita; Flynn, Corey; Rosains, Jacqueline; Takeda, David Y; Hu, Roger; Davison, Desiree; Lamb, Justin; Ardlie, Kristin; Hogstrom, Larson; Greenside, Peyton; Gray, Nathanael S; Clemons, Paul A; Silver, Serena; Wu, Xiaoyun; Zhao, Wen-Ning; Read-Button, Willis; Wu, Xiaohua; Haggarty, Stephen J; Ronco, Lucienne V; Boehm, Jesse S; Schreiber, Stuart L; Doench, John G; Bittker, Joshua A; Root, David E; Wong, Bang; & Golub, Todd R (2017). '**A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles.**' *Cell*. 171(6), pp. 1437-1452.e17.

Sun, et al., 2009 — Sun, Liou; Sadighi Akha, Amir A; Miller, Richard A; & Harper, James M (2009). '**Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 64(7), pp. 711-22.

Sun & Yi, 2015 Sun, Dan & Yi, Soojin V (2015). '**Impacts of Chromatin States and Long-Range Genomic Segments on Aging and DNA Methylation.**' *PloS one*. 10(6), pp. e0128517.

Suzuki, et al., 2010 Suzuki, Makoto; Willcox, D Craig; Rosenbaum, Matthew W; & Willcox, Bradley J (2010). '**Oxidative stress and longevity in okinawa: an investigation of blood lipid peroxidation and tocopherol in okinawan centenarians.**' *Current gerontology and geriatrics research*. 2010, pp. 380460.

Swindell, 2008a Swindell, William R (2008). '**Comparative analysis of microarray data identifies common responses to caloric restriction among mouse tissues.**' *Mechanisms of ageing and development*. 129(3), pp. 138-53.

Swindell, 2008b Swindell, William R (2008). '**Genes regulated by caloric restriction have unique roles within transcriptional networks.**' *Mechanisms of ageing and development*. 129(10), pp. 580-92.

Swindell, 2008c Swindell, William R (2008). '**Genes regulated by caloric restriction have unique roles within transcriptional networks.**' *Mechanisms of ageing and development*. 129(10), pp. 580-92.

Swindell, 2008d Swindell, William R (2008). '**Comparative analysis of microarray data identifies common responses to caloric restriction among mouse tissues.**' *Mechanisms of ageing and development*. 129(3), pp. 138-53.

Swindell, 2009 Swindell, William R (2009). '**Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse.**' *BMC genomics*. 10, pp. 585.

Swindell, 2012 Swindell, William R (2012). '**Dietary restriction in rats and mice: a meta-analysis and review of the evidence for genotype-dependent effects on lifespan.**' *Ageing research reviews*. 11(2), pp. 254-70.

Swindell, et al., 2012 Swindell, William R; Johnston, Andrew; Sun, Liou; Xing, Xianying; Fisher, Gary J; Bulyk, Martha L; Elder, James T; & Gudjonsson, Johann E (2012). '**Meta-profiles of gene expression during aging: limited similarities between mouse and human and an unexpectedly decreased inflammatory signature.**' *PloS one*. 7(3), pp. e33204.

Swindell, et al., 2018 Swindell WR, List EO, Berryman DE, Kopchick JJ. (2018) '**Transcriptional profiling identifies strain-specific effects of caloric restriction and opposite responses in human and mouse white adipose tissue.**' Aging (Albany NY). 2018 Apr 29;10(4):701-746. doi: 10.18632/aging.101424.

Szafranski & Mekhail, 2014 Szafranski, Kirk & Mekhail, Karim (2014). '**The fine line between lifespan extension and shortening in response to caloric restriction.**' *Nucleus (Austin, Tex.)*. 5(1), pp. 56-65.

Tabor, 1981 Tabor, C W (1981). '**Mutants of Saccharomyces cerevisiae deficient in polyamine biosynthesis: studies on the regulation of ornithine decarboxylase.**' *Medical biology*. 59(5-6), pp. 272-8.

Tacutu, et al., 2012 Tacutu, Robi; Shore, David E; Budovsky, Arie; de Magalhaes, Joao Pedro; Ruvkun, Gary; Fraifeld, Vadim E; & Curran, Sean P (2012). '**Prediction of C. elegans longevity genes by human and worm longevity networks.**' *PloS one*. 7(10), pp. e48282.

Tacutu, et al., 2013 Tacutu, Robi; Craig, Thomas; Budovsky, Arie; Wuttke, Daniel; Lehmann, Gilad; Taranukha, Dmitri; Costa, Joana; Fraifeld, Vadim E; & de Magalhaes, Joao Pedro (2013). '**Human Ageing Genomic Resources: integrated databases and tools for the biology and genetics of ageing.**' *Nucleic acids research*. 41(Database issue), pp. D1027-33.

Takahashi, et al., 1987 Takahashi, S; Gottschall, P E; Quigley, K L; Goya, R G; & Meites, J (1987). '**Growth hormone secretory patterns in young, middle-aged and old female rats.**' *Neuroendocrinology*. 46(2), pp. 137-42.

Takahashi, et al., 2007 Takahashi, Akiko; Ohtani, Naoko; Hara, Eiji (2007). **"Irreversibility of cellular senescence: dual roles of p16INK4a/Rb-pathway in cell cycle control."** Cell Div. 2007 Mar 7;2:10.

Takenaka, et al., 2000 Takenaka, A; Oki, N; Takahashi, S I; & Noguchi, T (2000). '**Dietary restriction of single essential amino acids reduces plasma insulin-like growth factor-I (IGF-I) but does not affect plasma IGF-binding protein-1 in rats.**' *The Journal of nutrition*. 130(12), pp. 2910-4.

Talwar, et al., 2014 Talwar, Puneet; Silla, Yumnam; Grover, Sandeep; Gupta, Meenal; Agarwal, Rachna; Kushwaha, Suman; & Kukreti, Ritushree (2014). '**Genomic convergence and network analysis approach to identify candidate genes in Alzheimer's disease.**' *BMC genomics*. 15, pp. 199.

Tang, et al., 2008 Tang, Fusheng; Watkins, Joseph W; Bermudez, Maria; Gray, Russell; Gaban, Adam; Portie, Ken; Grace, Stephen; Kleve, Maurice; & Craciun, Gheorghe (2008). '**A life-span extending form of autophagy employs the vacuole-vacuole fusion machinery.**' *Autophagy*. 4(7), pp. 874-86.

Tang & Choe, 2015 Tang, Lanlan; Choe, Keith P (2015). **"Characterization of skn-1/wdr-23 phenotypes in Caenorhabditis elegans; pleiotrophy, aging, glutathione, and interactions with other longevity pathways."** Mech Ageing Dev. 2015 Jul;149:88-98. doi: 10.1016/j.mad.2015.06.001. Epub 2015 Jun 6.

Tanrikulu-Kucuk & Ademoglu, 2012 Tanrikulu-Kucuk, Sevda & Ademoglu, Evin (2012). '**Dietary restriction of amino acids other than methionine prevents oxidative damage during aging: involvement of telomerase activity and telomere length.**' *Life sciences*. 90(23-24), pp. 924-8.

Tao, et al., 2004 Tao, Dan; Lu, Jun; Sun, Hui; Zhao, Yan-Mei; Yuan, Zhi-Gen; Li, Xiao-Xue; & Huang, Bai-Qu (2004). '**Trichostatin A extends the lifespan of Drosophila melanogaster by elevating hsp22 expression.**' *Acta biochimica et biophysica Sinica*. 36(9), pp. 618-22.

Tarcea, et al., 2009 Tarcea, V Glenn; Weymouth, Terry; Ade, Alex; Bookvich, Aaron; Gao, Jing; Mahavisno, Vasudeva; Wright, Zach; Chapman, Adriane; Jayapandian, Magesh; Ozgur, Arzucan; Tian, Yuanyuan; Cavalcoli, Jim; Mirel, Barbara; Patel, Jignesh; Radev, Dragomir; Athey, Brian; States, David; & Jagadish, H V (2009). '**Michigan molecular interactions r2: from interacting proteins to pathways.**' *Nucleic acids research*. 37(Database issue), pp. D642-6.

Tarpey & Mendoza, 1980 Tarpey, G M & Mendoza, S A (1980). '**Effects of clofibrate on water permeability and short-cicuit current of toad urinary bladder.**' *The Journal of pharmacology and experimental therapeutics*. 214(1), pp. 5-8.

Tazearslan, et al., 2009 Tazearslan, Cagdas; Ayyadevara, Srinivas; Bharill, Puneet; & Shmookler Reis, Robert J (2009). '**Positive feedback between transcriptional and kinase suppression in nematodes with extraordinary longevity and stress resistance.**' *PLoS genetics*. 5(4), pp. e1000452.

Tennen, et al., 2011 Tennen, Ruth I; Bua, Dennis J; Wright, Woodring E; & Chua, Katrin F (2011). '**SIRT6 is required for maintenance of telomere position effect in human cells.**' *Nature communications*. 2, pp. 433.

Thomas, 2001 Thomas, D R (2001). '**Age-related changes in wound healing.**' *Drugs & aging*. 18(8), pp. 607-20.

Thomas & Horvitz, 1999 Thomas, J H & Horvitz, H R (1999). '**The C. elegans gene lin-36 acts cell autonomously in the lin-35 Rb pathway.**' *Development (Cambridge, England)*. 126(15), pp. 3449-59.

Tian, et al., 2016 Tian, Ye; Garcia, Gilberto; Bian, Qian; Steffen, Kristan K; Joe, Larry; Wolff, Suzanne; Meyer, Barbara J; & Dillin, Andrew (2016). '**Mitochondrial Stress Induces Chromatin Reorganization to Promote Longevity and UPR(mt).**' *Cell*. 165(5), pp. 1197-208.

Tissenbaum & Guarente, 2001 Tissenbaum, H A & Guarente, L (2001). '**Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans.**' *Nature*. 410(6825), pp. 227-30.

Tocchetti, et al., 2010 — Tocchetti, Arianna; Soppo, Charlotte Blanche Ekalle; Zani, Fabio; Bianchi, Fabrizio; Gagliani, Maria Cristina; Pozzi, Benedetta; Rozman, Jan; Elvert, Ralf; Ehrhardt, Nicole; Rathkolb, Birgit; Moerth, Corinna; Horsch, Marion; Fuchs, Helmut; Gailus-Durner, Valerie; Beckers, Johannes; Klingenspor, Martin; Wolf, Eckhard; Hrabe de Angelis, Martin; Scanziani, Eugenio; Tacchetti, Carlo; Scita, Giorgio; Di Fiore, Pier Paolo; & Offenhauser, Nina (2010). '**Loss of the actin remodeler Eps8 causes intestinal defects and improved metabolic status in mice.**' *PloS one*. 5(3), pp. e9468.

Tocchi, et al., 2015 — Tocchi, Autumn; Quarles, Ellen K; Basisty, Nathan; Gitari, Lemuel; Rabinovitch, Peter S (2015). **"Mitochondrial dysfunction in cardiac aging."** Biochim Biophys Acta. 2015 Nov;1847(11):1424-33. doi: 10.1016/j.bbabio.2015.07.009. Epub 2015 Jul 17.

Tomaru, et al., 2012 — Tomaru, Utano; Takahashi, Satomi; Ishizu, Akihiro; Miyatake, Yukiko; Gohda, Aya; Suzuki, Sayuri; Ono, Ayako; Ohara, Jiro; Baba, Tomohisa; Murata, Shigeo; Tanaka, Keiji; & Kasahara, Masanori (2012). '**Decreased proteasomal activity causes age-related phenotypes and promotes the development of metabolic abnormalities.**' *The American journal of pathology*. 180(3), pp. 963-72.

Tothova, et al., 2007 — Tothova, Zuzana; Kollipara, Ramya; Huntly, Brian J; Lee, Benjamin H; Castrillon, Diego H; Cullen, Dana E; McDowell, Elizabeth P; Lazo-Kallanian, Suzan; Williams, Ifor R; Sears, Christopher; Armstrong, Scott A; Passegue, Emmanuelle; DePinho, Ronald A; & Gilliland, D Gary (2007). '**FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress.**' *Cell*. 128(2), pp. 325-39.

Trepanowski, et al., 2011 — Trepanowski, John F; Canale, Robert E; Marshall, Kate E; Kabir, Mohammad M; & Bloomer, Richard J (2011). '**Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings.**' *Nutrition journal*. 10, pp. 107.

Troen, et al., 2007 — Troen, Aron M; French, Emily E; Roberts, Jessica F; Selhub, Jacob; Ordovas, Jose M; Parnell, Laurence D; & Lai, Chao-Qiang (2007). '**Lifespan modification by glucose and methionine in Drosophila melanogaster fed a chemically defined diet.**' *Age (Dordrecht, Netherlands)*. 29(1), pp. 29-39.

Tsai, et al., 2012 — Tsai, Pei-Chien; Spector, Tim D; & Bell, Jordana T (2012). '**Using epigenome-wide association scans of DNA methylation in age-related complex human traits.**' *Epigenomics*. 4(5), pp. 511-26.

Uddin & Singh, 2013 — Uddin, Raihan K & Singh, Shiva M (2013). '**Hippocampal gene expression meta-analysis identifies aging and age-associated spatial learning impairment (ASLI) genes and pathways.**' *PloS one*. 8(7), pp. e69768.

Umebayashi & Nakano, 2003 — Umebayashi, Kyohei & Nakano, Akihiko (2003). '**Ergosterol is required for targeting of tryptophan permease to the yeast plasma membrane.**' *The Journal of cell biology*. 161(6), pp. 1117-31.

Unal, et al., 2011 — Unal, Elcin; Kinde, Benyam; & Amon, Angelika (2011). '**Gametogenesis eliminates age-induced cellular damage and resets life span in yeast.**' *Science (New York, N.Y.)*. 332(6037), pp. 1554-7.

Unal & Amon, 2011 — Unal, E & Amon, A (2011). '**Gamete formation resets the aging clock in yeast.**' *Cold Spring Harbor symposia on quantitative biology*. 76, pp. 73-80.

Ungewitter & Scrable, 2009 — Ungewitter, Erica; Scrable, Heidi (2009). **"Antagonistic pleiotropy and p53."** Mech Ageing Dev. 2009 Jan-Feb;130(1-2):10-7. doi: 10.1016/j.mad.2008.06.002. Epub 2008 Jul 1.

Unknown, 2008 — Unknown (2008). **"The role of mitochondria in aging."** Biotechnol J. 2008 Jun;3(6):708-10. doi: 10.1002/biot.200890065.

Urbanelli, et al., 2016 — Urbanelli, Lorena; Buratta, Sandra; Sagini, Krizia; Tancini, Brunella; Emiliani, Carla (2016). **"Extracellular Vesicles as New Players in Cellular Senescence."** Int J Mol Sci. 2016 Aug 26;17(9). pii: E1408. doi: 10.3390/ijms17091408.

Vaiserman, 2014 — Vaiserman, Alexander M (2014). '**Aging-modulating treatments: from reductionism to a system-oriented perspective.**' *Frontiers in genetics*. 5, pp. 446.

Vaiserman, et al., 2012 Vaiserman, A M; Koliada, A K; Koshel', N M; Simonenko, A V; & Pasiukova, E G (2012). '**[Effect of the histone deacetylase inhibitor sodium butyrate on the viability and life span in Drosophila melanogaster].**' *Advances in gerontology = Uspekhi gerontologii*. 25(1), pp. 126-31.

Vaiserman, et al., 2013 Vaiserman, A M; Koliada, A K; & Zabuga, O G (2013). '**[Effect of dietary restriction during development on the level of expression of longevity-associated genes in Drosophila melanogaster].**' *Advances in gerontology = Uspekhi gerontologii*. 26(4), pp. 638-42.

Vaiserman, et al., 2017 Alexander M Vaiserman, David Gems, Eric Le Bourg Alexander N Khokhlov Ramon Cacabelos, Francesco Marotta, Suresh I.S. Rattan, Vitaly Koltover, Vladimir P Skulachev, Oleh Lushchak, Basil P Hubbard, Francisco J Alcain, Kyung-Jin Min, Dudley Lamming, Zelton David Sharp, H Y Chung, Vladimir N Anisimov, Wil A M Loenen, Rudiger Hardeland, Vladimir Khavinson, E. G. Pasyukova, Ilia Stambler, David Thurston, Ana Martinez (2017) '**Anti-aging Drugs: From Basic Research to Clinical.**' Practice Hardcover – Feb 1 2017

Valentini, 2009 **'Giorgio Valentini True Path Rule Hierarchical Ensembles'**

Van Bussel, et al., 2016 Van Bussel, I P G; Jolink-Stoppelenburg, A; De Groot, C P G M; Muller, M R; & Afman, L A (2016). '**Differences in genome-wide gene expression response in peripheral blood mononuclear cells between young and old men upon caloric restriction.**' *Genes & nutrition*. 11, pp. 13.

van den Akker, et al., 2014 van den Akker, Erik B; Passtoors, Willemijn M; Jansen, Rick; van Zwet, Erik W; Goeman, Jelle J; Hulsman, Marc; Emilsson, Valur; Perola, Markus; Willemsen, Gonneke; Penninx, Brenda W J H; Heijmans, Bas T; Maier, Andrea B; Boomsma, Dorret I; Kok, Joost N; Slagboom, Pieternella E; Reinders, Marcel J T; & Beekman, Marian (2014). '**Meta-analysis on blood transcriptomic studies identifies consistently coexpressed protein-protein interaction modules as robust markers of human aging.**' *Aging cell*. 13(2), pp. 216-25.

van Heemst, 2010 van Heemst, Diana (2010). '**Insulin, IGF-1 and longevity.**' *Aging and disease*. 1(2), pp. 147-57.

Van Neste & Tobin, 2004 Van Neste, Dominique & Tobin, Desmond J (2004). '**Hair cycle and hair pigmentation: dynamic interactions and changes associated with aging.**' *Micron (Oxford, England : 1993)*. 35(3), pp. 193-200.

Vaquero & Reinberg, 2009 Vaquero, Alejandro & Reinberg, Danny (2009). '**Calorie restriction and the exercise of chromatin.**' *Genes & development*. 23(16), pp. 1849-69.

Vasan, et al., 2001 Vasan, S; Foiles, P G; & Founds, H W (2001). '**Therapeutic potential of AGE inhibitors and breakers of AGE protein cross-links.**' *Expert opinion on investigational drugs*. 10(11), pp. 1977-87.

Vasiliu, et al., 2015 Vasiliu, Daniel; Clamons, Samuel; McDonough, Molly; Rabe, Brian; & Saha, Margaret (2015). '**A regression-based differential expression detection algorithm for microarray studies with ultra-low sample size.**' *PloS one*. 10(3), pp. e0118198.

Venter, et al., 2001 Venter, J C, et Al (2001). '**The sequence of the human genome.**' *Science (New York, N.Y.)*. 291(5507), pp. 1304-51.

Vera, et al., 2013 Vera, Elsa; Bernardes de Jesus, Bruno; Foronda, Miguel; Flores, Juana M; & Blasco, Maria A (2013). '**Telomerase reverse transcriptase synergizes with calorie restriction to increase health span and extend mouse longevity.**' *PloS one*. 8(1), pp. e53760.

Verbeke, et al., 2000 Verbeke, P; Clark, B F; Rattan, S I (2000). **"Modulating cellular aging in vitro: hormetic effects of repeated mild heat stress on protein oxidation and glycation."** Exp Gerontol. 2000 Sep;35(6-7):787-94.

Vigano, et al., 2006 Vigano, M Alessandra; Lamartine, Jerome; Testoni, Barbara; Merico, Daniele; Alotto, Daniela; Castagnoli, Carlotta; Robert, Amelie; Candi, Eleonora; Melino, Gerry; Gidrol, Xavier; & Mantovani, Roberto (2006). '**New p63 targets in keratinocytes identified by a genome-wide approach.**' *The EMBO journal*. 25(21), pp. 5105-16.

Vijg, 2004 Vijg, Jan (2004). '**Impact of genome instability on transcription regulation of aging and senescence.**' *Mechanisms of ageing and development*. 125(10-11), pp. 747-53.

Visser, et al., 2016 Visser, W Edward; Bombardieri, Cintia R; Zevenbergen, Chantal; Barnhoorn, Sander; Ottaviani, Alexandre; van der Pluijm, Ingrid; Brandt, Renata; Kaptein, Ellen; van Heerebeek, Ramona; van Toor, Hans; Garinis, George A; Peeters, Robin P; Medici, Marco; van Ham, Willy; Vermeij, Wilbert P; de Waard, Monique C; de Krijger, Ronald R; Boelen, Anita; Kwakkel, Joan; Kopchick, John J; List, Edward O; Melis, Joost P M; Darras, Veerle M; Dolle, Martijn E T; van der Horst, Gijsbertus T J; Hoeijmakers, Jan H J; & Visser, Theo J (2016). '**Tissue-Specific Suppression of Thyroid Hormone Signaling in Various Mouse Models of Aging.**' *PloS one*. 11(3), pp. e0149941.

Vora, et al., 2013 Vora, Mehul; Shah, Mitalie; Ostafi, Silvana; Onken, Brian; Xue, Jian; Ni, Julie Zhouli; Gu, Sam; & Driscoll, Monica (2013). '**Deletion of microRNA-80 activates dietary restriction to extend C. elegans healthspan and lifespan.**' *PLoS genetics*. 9(8), pp. e1003737.

Voutetakis, et al., 2015 Voutetakis, Konstantinos; Chatziioannou, Aristotelis; Gonos, Efstathios S; & Trougakos, Ioannis P (2015). '**Comparative Meta-Analysis of Transcriptomics Data during Cellular Senescence and In Vivo Tissue Ageing.**' *Oxidative medicine and cellular longevity*. 2015, pp. 732914.

Wachsman, 1996 Wachsman, J T (1996). '**The beneficial effects of dietary restriction: reduced oxidative damage and enhanced apoptosis.**' *Mutation research*. 350(1), pp. 25-34.

Walford, et al., 1992 Walford, R L; Harris, S B; & Gunion, M W (1992). '**The calorically restricted low-fat nutrient-dense diet in Biosphere 2 significantly lowers blood glucose, total leukocyte count, cholesterol, and blood pressure in humans.**' *Proceedings of the National Academy of Sciences of the United States of America*. 89(23), pp. 11533-7.

Walford, et al., 2002 Walford, Roy L; Mock, Dennis; Verdery, Roy; & MacCallum, Taber (2002). '**Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 57(6), pp. B211-24.

Walhout, et al., 2000 Walhout, A J; Sordella, R; Lu, X; Hartley, J L; Temple, G F; Brasch, M A; Thierry-Mieg, N; & Vidal, M (2000). '**Protein interaction mapping in C. elegans using proteins involved in vulval development.**' *Science (New York, N.Y.)*. 287(5450), pp. 116-22.

Walker & Lithgow, 2003 Walker, Glenda A & Lithgow, Gordon J (2003). '**Lifespan extension in C. elegans by a molecular chaperone dependent upon insulin-like signals.**' *Aging cell*. 2(2), pp. 131-9.

Walter & Ron, 2011 Walter, Peter & Ron, David (2011). '**The unfolded protein response: from stress pathway to homeostatic regulation.**' *Science (New York, N.Y.)*. 334(6059), pp. 1081-6.

Wan, et al., 2015 Wan, Cen; Freitas, Alex A; de Magalhaes, Joao Pedro (2015). **"Predicting the Pro-Longevity or Anti-Longevity Effect of Model Organism Genes with New Hierarchical Feature Selection Methods."** IEEE/ACM Trans Comput Biol Bioinform. 2015 Mar-Apr;12(2):262-75. doi: 10.1109/TCBB.2014.2355218.

Wang, 1995 Wang, E (1995). **"Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved."** Cancer Res. 1995 Jun 1;55(11):2284-92.

Wang, 2012 Wang, Xiaosheng (2012). '**Microarray analysis of ageing-related signatures and their expression in tumors based on a computational biology approach.**' *Genomics, proteomics & bioinformatics*. 10(3), pp. 136-41.

Wang, 2013 Wang, Xiaosheng (2013). '**Discovery of molecular associations among aging, stem cells, and cancer based on gene expression profiling.**' *Chinese journal of cancer*. 32(4), pp. 155-61.

Wang, et al., 1998 Wang, Y X; Catlett, N L; & Weisman, L S (1998). '**Vac8p, a vacuolar protein with armadillo repeats, functions in both vacuole inheritance and protein targeting from the cytoplasm to vacuole.**' *The Journal of cell biology*. 140(5), pp. 1063-74.

Wang, et al., 2009 Wang, Zhong Q; Floyd, Z Elizabeth; Qin, Jianhua; Liu, Xiaotuan; Yu, Yongmei; Zhang, Xian H; Wagner, Janice D; & Cefalu, William T (2009). '**Modulation of skeletal muscle insulin signaling with chronic caloric restriction in cynomolgus monkeys.**' *Diabetes*. 58(7), pp. 1488-98.

Wang, et al., 2009b — Wang, Chunfang; Jurk, Diana; Maddick, Mandy; Nelson, Glyn; Martin-Ruiz, Carmen; & von Zglinicki, Thomas (2009). '**DNA damage response and cellular senescence in tissues of aging mice.**' *Aging cell*. 8(3), pp. 311-23.

Wang, et al., 2010 — Wang, Chunfang; Maddick, Mandy; Miwa, Satomi; Jurk, Diana; Czapiewski, Rafal; Saretzki, Gabriele; Langie, Sabine A S; Godschalk, Roger W L; Cameron, Kerry; von Zglinicki, Thomas (2010). **"Adult-onset, short-term dietary restriction reduces cell senescence in mice."** Aging (Albany NY). 2010 Sep;2(9):555-66.

Wang, et al., 2012 — Wang, Li; Yang, Wu; Ju, Weina; Wang, Peirong; Zhao, Xinliang; Jenkins, Edmund C; Brown, W Ted; Zhong, Nanbert (2012). **"A proteomic study of Hutchinson-Gilford progeria syndrome: Application of 2D-chromotography in a premature aging disease."** Biochem Biophys Res Commun. 2012 Jan 27;417(4):1119-26. doi: 10.1016/j.bbrc.2011.12.056. Epub 2011 Dec 24.

Wang, et al., 2013 — Wang, Shuai; Hannafon, Bethany N; Zhou, Jundong; & Ding, Wei-Qun (2013). '**Clofibrate induces heme oxygenase 1 expression through a PPARalpha-independent mechanism in human cancer cells.**' *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology*. 32(5), pp. 1255-64.

Wang, et al., 2015 — Wang, Jiasi; Zhao, Chuanqi; Zhao, Andong; Li, Meng; Ren, Jinsong; & Qu, Xiaogang (2015). '**New insights in amyloid beta interactions with human telomerase.**' *Journal of the American Chemical Society*. 137(3), pp. 1213-9.

Wang, et al., 2017 — Wang, Tina; Tsui, Brian; Kreisberg, Jason F; Robertson, Neil A; Gross, Andrew M; Yu, Michael Ku; Carter, Hannah; Brown-Borg, Holly M; Adams, Peter D; & Ideker, Trey (2017). '**Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment.**' *Genome biology*. 18(1), pp. 57.

Wang & Gundersen, 1984 — Wang, E & Gundersen, D (1984). '**Increased organization of cytoskeleton accompanying the aging of human fibroblasts in vitro.**' *Experimental cell research*. 154(1), pp. 191-202.

Warner, 1999 — Warner, H R (1999). '**Apoptosis: a two-edged sword in aging.**' *Anticancer research*. 19(4B), pp. 2837-42.

Warsow, et al., 2010 — Warsow, Gregor; Greber, Boris; Falk, Steffi S I; Harder, Clemens; Siatkowski, Marcin; Schordan, Sandra; Som, Anup; Endlich, Nicole; Scholer, Hans; Repsilber, Dirk; Endlich, Karlhans; & Fuellen, Georg (2010). '**ExprEssence--revealing the essence of differential experimental data in the context of an interaction/regulation net-work.**' *BMC systems biology*. 4, pp. 164.

Wehrspaun, et al., 2015 — Wehrspaun, Claudia C; Haerty, Wilfried; & Ponting, Chris P (2015). '**Microglia recapitulate a hematopoietic master regulator network in the aging human frontal cortex.**' *Neurobiology of aging*. 36(8), pp. 2443.e9-2443.e20.

Wei, et al., 2008 — Wei M, Fabrizio P, Hu J, Ge H, Cheng C, Li L, Longo VD (2008) **Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9.**. *PLoS genetics*, 4(1), pp.e13.

Wei, et al., 2015 — Wei, Yu-Ning; Hu, Hai-Yang; Xie, Gang-Cai; Fu, Ning; Ning, Zhi-Bin; Zeng, Rong; & Khaitovich, Philipp (2015). '**Transcript and protein expression decoupling reveals RNA binding proteins and miRNAs as potential modulators of human aging.**' *Genome biology*. 16, pp. 41.

Weindruch, 1992 — Weindruch, R (1992). '**Effect of caloric restriction on age-associated cancers.**' *Experimental gerontology*. 27(5-6), pp. 575-81.

Weindruch, et al., 1980 — Weindruch, R H; Cheung, M K; Verity, M A; & Walford, R L (1980). '**Modification of mitochondrial respiration by aging and dietary restriction.**' *Mechanisms of ageing and development*. 12(4), pp. 375-92.

Weindruch, et al., 1986 — Weindruch, R; Walford, R L; Fligiel, S; & Guthrie, D (1986). '**The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake.**' *The Journal of nutrition*. 116(4), pp. 641-54.

Weng, et al., 2014 Weng, Matthias K; Natarajan, Karthick; Scholz, Diana; Ivanova, Violeta N; Sachinidis, Agapios; Hengstler, Jan G; Waldmann, Tanja; Leist, Marcel (2014). **"Lineage-specific regulation of epigenetic modifier genes in human liver and brain."** PLoS One. 2014 Jul 23;9(7):e102035. doi: 10.1371/journal.pone.0102035. eCollection 2014.

Westbrook, et al., 2014 Westbrook, Reyhan; Bonkowski, Michael S; Arum, Oge; Strader, April D; & Bartke, Andrzej (2014). '**Metabolic alterations due to caloric restriction and every other day feeding in normal and growth hormone receptor knockout mice.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 69(1), pp. 25-33.

Whitlock, 2005 Whitlock, M C (2005). '**Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach.**' *Journal of evolutionary biology*. 18(5), pp. 1368-73.

Wiles, et al., 2010 Wiles, Amy M; Doderer, Mark; Ruan, Jianhua; Gu, Ting-Ting; Ravi, Dashnamoorthy; Blackman, Barron; & Bishop, Alexander J R (2010). '**Building and analyzing protein interactome networks by cross-species comparisons.**' *BMC systems biology*. 4, pp. 36.

Willcox, et al., 2006a Willcox, Bradley J; Willcox, D Craig; He, Qimei; Curb, J David; & Suzuki, Makoto (2006). '**Siblings of Okinawan centenarians share lifelong mortality advantages.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 61(4), pp. 345-54.

Willcox, et al., 2006b Willcox, D Craig; Willcox, Bradley J; Todoriki, Hidemi; Curb, J David; & Suzuki, Makoto (2006). '**Caloric restriction and human longevity: what can we learn from the Okinawans?**' *Biogerontology*. 7(3), pp. 173-7.

Willcox, et al., 2007a Willcox, Bradley J; Willcox, D Craig; Todoriki, Hidemi; Fujiyoshi, Akira; Yano, Katsuhiko; He, Qimei; Curb, J David; & Suzuki, Makoto (2007). '**Caloric restriction, the traditional Okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span.**' *Annals of the New York Academy of Sciences*. 1114, pp. 434-55.

Willcox, et al., 2007b Willcox, D Craig; Willcox, Bradley J; Shimajiri, Sanae; Kurechi, Sayuri; & Suzuki, Makoto (2007). '**Aging gracefully: a retrospective analysis of functional status in Okinawan centenarians.**' *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry*. 15(3), pp. 252-6.

Willcox, et al., 2008a Willcox, D Craig; Willcox, Bradley J; He, Qimei; Wang, Nien-chiang; & Suzuki, Makoto (2008). '**They really are that old: a validation study of centenarian prevalence in Okinawa.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 63(4), pp. 338-49.

Willcox, et al., 2008b Willcox, D Craig; Willcox, Bradley J; Wang, Nien-Chiang; He, Qimei; Rosenbaum, Matthew; & Suzuki, Makoto (2008). '**Life at the extreme limit: phenotypic characteristics of supercentenarians in Okinawa.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 63(11), pp. 1201-8.

Willcox & Willcox, 2014 Willcox, Bradley J & Willcox, Donald C (2014). '**Caloric restriction, caloric restriction mimetics, and healthy aging in Okinawa: controversies and clinical implications.**' *Current opinion in clinical nutrition and metabolic care*. 17(1), pp. 51-8.

Williams, 2014 Williams, Chris (2014). **"Going against the flow: a case for peroxisomal protein export."** Biochim Biophys Acta. 2014 Jul;1843(7):1386-92. doi: 10.1016/j.bbamcr.2014.04.009. Epub 2014 Apr 15.

Wilson, et al., 1995 Wilson, W H; Jamis-Dow, C; Bryant, G; Balis, F M; Klecker, R W; Bates, S E; Chabner, B A; Steinberg, S M; Kohler, D R; & Wittes, R E (1995). '**Phase I and pharmacokinetic study of the multidrug resistance modulator dexverapamil with EPOCH chemotherapy.**' *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 13(8), pp. 1985-94.

Witten & Bonchev, 2007 Witten, Tarynn M & Bonchev, Danail (2007). '**Predicting aging/longevity-related genes in the nematode Caenorhabditis elegans.**' *Chemistry & biodiversity*. 4(11), pp. 2639-55.

Wood, et al., 2010 Wood, Jason G; Hillenmeyer, Sara; Lawrence, Charles; Chang, Chengyi; Hosier, Suzanne; Lightfoot, Will; Mukherjee, Eric; Jiang, Nan; Schorl, Christoph; Brodsky, Alexander S; Neretti, Nicola; & Helfand, Stephen L (2010). '**Chromatin remodeling in the aging genome of Drosophila.**' *Aging cell*. 9(6), pp. 971-8.

Wood, et al., 2016 Wood, Jason G; Jones, Brian C; Jiang, Nan; Chang, Chengyi; Hosier, Suzanne; Wickremesinghe, Priyan; Garcia, Meyrolin; Hartnett, Davis A; Burhenn, Lucas; Neretti, Nicola; & Helfand, Stephen L (2016). '**Chromatin-modifying genetic interventions suppress age-associated transposable element activation and extend life span in Drosophila.**' *Proceedings of the National Academy of Sciences of the United States of America*. 113(40), pp. 11277-11282.

Wu, et al., 2009b Wu, Jianmin; Vallenius, Tea; Ovaska, Kristian; Westermarck, Jukka; Makela, Tomi P; & Hautaniemi, Sampsa (2009). '**Integrated network analysis platform for protein-protein interactions.**' *Nature methods*. 6(1), pp. 75-7.

Wu, et al., 2013 Wu, Ziyun; Song, Lixia; Liu, Shao Quan; & Huang, Dejian (2013). '**Independent and additive effects of glutamic acid and methionine on yeast longevity.**' *PloS one*. 8(11), pp. e79319.

Wu & Yan, 2011 Wu, L; Yan, B (2011). **"Discovery of small molecules that target autophagy for cancer treatment."** Curr Med Chem. 2011;18(12):1866-73.

Wuttke, et al., 2012 Wuttke D, Connor R, Vora C, Craig T, Li Y, Wood S, Vasieva O, Shmookler Reis R, Tang F, de Magalhaes JP (2012) **Dissecting the gene network of dietary restriction to identify evolutionarily conserved pathways and new functional genes.**. *PLoS genetics*, 8(8), pp.e1002834. source: PLoS Genet. 2012;8(8):e1002834. doi: 10.1371/journal.pgen.1002834. Epub 2012 Aug 9.

Wuttke & de Magalhaes, 2011 Wuttke, Daniel & de Magalhães, Joao Pedro (2011) '**Molecular Signatures to Decipher Ageing**'. *Reproductive ageing: a basic clinical update*. Book chapter.

Wuttke & de Magalhaes, 2012 Wuttke, Daniel & de Magalhaes, Joao Pedro (2012). '**Osh6 links yeast vacuolar functions to lifespan extension and TOR.**' *Cell cycle (Georgetown, Tex.)*. 11(13), pp. 2419.

Xenarios, et al., 2002 Xenarios, Ioannis; Salwinski, Lukasz; Duan, Xiaoqun Joyce; Higney, Patrick; Kim, Sul-Min; & Eisenberg, David (2002). '**DIP, the Database of Interacting Proteins: a research tool for studying cellular networks of protein interactions.**' *Nucleic acids research*. 30(1), pp. 303-5.

Xu, et al., 2005 Xu, Feng; Zhang, Kangling; & Grunstein, Michael (2005). '**Acetylation in histone H3 globular domain regulates gene expression in yeast.**' *Cell*. 121(3), pp. 375-85.

Xue, et al., 1998 Xue, Y; Wong, J; Moreno, G T; Young, M K; Cote, J; & Wang, W (1998). '**NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities.**' *Molecular cell*. 2(6), pp. 851-61.

Xue, et al., 2007 Xue, Wen; Zender, Lars; Miething, Cornelius; Dickins, Ross A; Hernando, Eva; Krizhanovsky, Valery; Cordon-Cardo, Carlos; & Lowe, Scott W (2007). '**Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas.**' *Nature*. 445(7128), pp. 656-60.

Yamaza, et al., 2010 Yamaza, Haruyoshi; Komatsu, Toshimitsu; Wakita, Saori; Kijogi, Carole; Park, Seongjoon; Hayashi, Hiroko; Chiba, Takuya; Mori, Ryoichi; Furuyama, Tatsuo; Mori, Nozomu; & Shimokawa, Isao (2010). '**FoxO1 is involved in the antineoplastic effect of calorie restriction.**' *Aging cell*. 9(3), pp. 372-82.

Yang, et al., 2015 Yang, Jialiang; Huang, Tao; Petralia, Francesca; Long, Quan; Zhang, Bin; Argmann, Carmen; Zhao, Yong; Mobbs, Charles V; Schadt, Eric E; Zhu, Jun; & Tu, Zhidong (2015). '**Synchronized age-related gene expression changes across multiple tissues in human and the link to complex diseases.**' *Scientific reports*. 5, pp. 15145.

Yao, et al., 2015 — Yao, Yanhua; Tsuchiyama, Scott; Yang, Ciyu; Bulteau, Anne Laure; He, Chong; Robison, Brett; Tsuchiya, Mitsuhiro; Miller, Delana; Briones, Valeria; Tar, Krisztina; Potrero, Anahi; Friguet, Bertrand; Kennedy, Brian K; & Schmidt, Marion (2015). '**Proteasomes, Sir2, and Hxk2 form an interconnected aging network that impinges on the AMPK/Snf1-regulated transcriptional repressor Mig1.**' *PLoS genetics*. 11(1), pp. e1004968.

Yau, et al., 2007 — Yau, Christina; Fedele, Vita; Roydasgupta, Ritu; Fridlyand, Jane; Hubbard, Alan; Gray, Joe W; Chew, Karen; Dairkee, Shanaz H; Moore, Dan H; Schittulli, Francesco; Tommasi, Stefania; Paradiso, Angelo; Albertson, Donna G; & Benz, Christopher C (2007). '**Aging impacts transcriptomes but not genomes of hormone-dependent breast cancers.**' *Breast cancer research : BCR*. 9(5), pp. R59.

Ye & Bhalla, 2011 — Ye, Alice L; Bhalla, Needhi (2011). **"Reproductive aging: insights from model organisms."** Biochem Soc Trans. 2011 Dec;39(6):1770-4. doi: 10.1042/BST20110694.

Ye & Keller, 2010 — Ye, Jianping & Keller, Jeffrey N (2010). '**Regulation of energy metabolism by inflammation: a feedback response in obesity and calorie restriction.**' *Aging*. 2(6), pp. 361-8.

Yen & Soong, 1996 — Yen, A & Soong, S (1996). '**Retinoic acid-induced RB (retinoblastoma) hypophosphorylation enhanced by CGP 52411 (4,5-dianilinophthalimide), an EGF family tyrosine kinase receptor inhibitor.**' *European journal of cell biology*. 69(4), pp. 327-34.

Yoshida, et al., 2007 — Yoshida, K; Shinohara, H; Haneji, T; & Nagata, T (2007). '**Arachidonic acid inhibits osteoblast differentiation through cytosolic phospholipase A2-dependent pathway.**' *Oral diseases*. 13(1), pp. 32-9.

YourTribute, 2013 — '**How will You Die? The Causes of Death in the US**' Available from: http://visual.ly/how-will-you-die-causes-death-us?(). [shared by shanemarks3 on Jun 04, 2013 in Health]

Yu, et al., 1985 — Yu, B P; Masoro, E J; & McMahan, C A (1985). '**Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics.**' *Journal of gerontology*. 40(6), pp. 657-70.

Yu, et al., 2014 — Yu, Peiwen; Laird, A Douglas; Du, Xiangnan; Wu, Jianming; Won, Kwang-Ai; Yamaguchi, Kyoko; Hsu, Pin Pin; Qian, Fawn; Jaeger, Christopher T; Zhang, Wentao; Buhr, Chris A; Shen, Paula; Abulafia, Wendy; Chen, Jason; Young, Jenny; Plonowski, Arthur; Yakes, F Michael; Chu, Felix; Lee, Michelle; Bentzien, Frauke; Lam, Sanh Tan; Dale, Stephanie; Matthews, David J; Lamb, Peter; & Foster, Paul (2014). '**Characterization of the activity of the PI3K/mTOR inhibitor XL765 (SAR245409) in tumor models with diverse genetic alterations affecting the PI3K pathway.**' *Molecular cancer therapeutics*. 13(5), pp. 1078-91.

Yuan, et al., 2012 — Yuan, Yuan; Chen, Yi-Ping Phoebe; Boyd-Kirkup, Jerome; Khaitovich, Philipp; & Somel, Mehmet (2012). '**Accelerated aging-related transcriptome changes in the female prefrontal cortex.**' *Aging cell*. 11(5), pp. 894-901.

Zahn, et al., 2006 — Zahn, Jacob M; Sonu, Rebecca; Vogel, Hannes; Crane, Emily; Mazan-Mamczarz, Krystyna; Rabkin, Ralph; Davis, Ronald W; Becker, Kevin G; Owen, Art B; & Kim, Stuart K (2006). '**Transcriptional profiling of aging in human muscle reveals a common aging signature.**' *PLoS genetics*. 2(7), pp. e115.

Zahn, et al., 2007 — Zahn, Jacob M; Poosala, Suresh; Owen, Art B; Ingram, Donald K; Lustig, Ana; Carter, Arnell; Weeraratna, Ashani T; Taub, Dennis D; Gorospe, Myriam; Mazan-Mamczarz, Krystyna; Lakatta, Edward G; Boheler, Kenneth R; Xu, Xiangru; Mattson, Mark P; Falco, Geppino; Ko, Minoru S H; Schlessinger, David; Firman, Jeffrey; Kummerfeld, Sarah K; Wood, William H 3rd; Zonderman, Alan B; Kim, Stuart K; & Becker, Kevin G (2007). '**AGEMAP: a gene expression database for aging in mice.**' *PLoS genetics*. 3(11), pp. e201.

Zahn & Kim, 2007 — Zahn, Jacob M & Kim, Stuart K (2007). '**Systems biology of aging in four species.**' *Current opinion in biotechnology*. 18(4), pp. 355-9.

Zhang, et al., 1999 Zhang, T; Xiong, H; Kan, L X; Zhang, C K; Jiao, X F; Fu, G; Zhang, Q H; Lu, L; Tong, J H; Gu, B W; Yu, M; Liu, J X; Licht, J; Waxman, S; Zelent, A; Chen, E; & Chen, S J (1999). '**Genomic sequence, structural organization, molecular evolution, and aberrant rearrangement of promyelocytic leukemia zinc finger gene.**' *Proceedings of the National Academy of Sciences of the United States of America*. 96(20), pp. 11422-7.

Zhang, et al., 2002 Zhang, Hannah J; Drake, Victoria J; Morrison, Joanna P; Oberley, Larry W; Kregel, Kevin C (2002). **"Selected contribution: Differential expression of stress-related genes with aging and hyperthermia."** J Appl Physiol (1985). 2002 Apr;92(4):1762-9; discussion 1749.

Zhang, et al., 2007 Zhang, Le; Li, Feng; Dimayuga, Edgardo; Craddock, Jeffrey; & Keller, Jeffrey N (2007). '**Effects of aging and dietary restriction on ubiquitination, sumoylation, and the proteasome in the spleen.**' *FEBS letters*. 581(28), pp. 5543-7.

Zhang, et al., 2011 Zhang, Jinqiu; Lian, Qizhou; Zhu, Guili; Zhou, Fan; Sui, Lin; Tan, Cindy; Mutalif, Rafidah Abdul; Navasankari, Raju; Zhang, Yuelin; Tse, Hung-Fat; Stewart, Colin L; Colman, Alan (2011). **"A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects."** Cell Stem Cell. 2011 Jan 7;8(1):31-45. doi: 10.1016/j.stem.2010.12.002. Epub 2010 Dec 23.

Zhang & Cuervo, 2008 Zhang, Cong & Cuervo, Ana Maria (2008). '**Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function.**' *Nature medicine*. 14(9), pp. 959-65.

Zhang & Yinghua, 2010 Zhang Y; Yinghua L (2010). '**The Expanding Mi-2/NuRD Complexes: A Schematic Glance.**' *Proteomics Insights*. 3: 79–109. doi:10.4137/PRI.S6329.

Zhao, et al., 2005 Zhao, Yanmei; Sun, Hui; Lu, Jun; Li, Xiaoxue; Chen, Xia; Tao, Dan; Huang, Weifeng; & Huang, Baiqu (2005). '**Lifespan extension and elevated hsp gene expression in Drosophila caused by histone deacetylase inhibitors.**' *The Journal of experimental biology*. 208(Pt 4), pp. 697-705.

Zhao, et al., 2014 Zhao, Guodong; Guo, Song; Somel, Mehmet; & Khaitovich, Philipp (2014). '**Evolution of human longevity uncoupled from caloric restriction mechanisms.**' *PloS one*. 9(1), pp. e84117.

Zhavoronkov, et al., 2014 Zhavoronkov, Alex; Buzdin, Anton A; Garazha, Andrey V; Borisov, Nikolay M; & Moskalev, Alexey A (2014). '**Signaling pathway cloud regulation for in silico screening and ranking of the potential geroprotective drugs.**' *Frontiers in genetics*. 5, pp. 49.

Zhen, et al., 1999 Zhen, X; Uryu, K; Cai, G; Johnson, G P; & Friedman, E (1999). '**Age-associated impairment in brain MAPK signal pathways and the effect of caloric restriction in Fischer 344 rats.**' *The journals of gerontology. Series A, Biological sciences and medical sciences*. 54(12), pp. B539-48.

Zhou, et al., 1997 Zhou, Y; Xu, B C; Maheshwari, H G; He, L; Reed, M; Lozykowski, M; Okada, S; Cataldo, L; Coschigamo, K; Wagner, T E; Baumann, G; & Kopchick, J J (1997). '**A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse).**' *Proceedings of the National Academy of Sciences of the United States of America*. 94(24), pp. 13215-20.

Zhu, et al., 2015 Zhu, Yi; Tchkonia, Tamara; Pirtskhalava, Tamar; Gower, Adam C; Ding, Husheng; Giorgadze, Nino; Palmer, Allyson K; Ikeno, Yuji; Hubbard, Gene B; Lenburg, Marc; O'Hara, Steven P; LaRusso, Nicholas F; Miller, Jordan D; Roos, Carolyn M; Verzosa, Grace C; LeBrasseur, Nathan K; Wren, Jonathan D; Farr, Joshua N; Khosla, Sundeep; Stout, Michael B; McGowan, Sara J; Fuhrmann-Stroissnigg, Heike; Gurkar, Aditi U; Zhao, Jing; Colangelo, Debora; Dorronsoro, Akaitz; Ling, Yuan Yuan; Barghouthy, Amira S; Navarro, Diana C; Sano, Tokio; Robbins, Paul D; Niedernhofer, Laura J; Kirkland, James L (2015). **"The Achilles' heel of senescent cells: from transcriptome to senolytic drugs."** Aging Cell. 2015 Aug;14(4):644-58. doi: 10.1111/acel.12344. Epub 2015 Apr 22.

Zhu, et al., 2016 Zhu, Yi; Tchkonia, Tamara; Fuhrmann-Stroissnigg, Heike; Dai, Haiming M; Ling, Yuanyuan Y; Stout, Michael B; Pirtskhalava, Tamar; Giorgadze, Nino; Johnson, Kurt O; Giles, Cory B; Wren, Jonathan D; Niedernhofer, Laura J; Robbins, Paul D; Kirkland, James L (2016). **"Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors."** Aging Cell. 2016 Jun;15(3):428-35. doi: 10.1111/acel.12445. Epub 2016 Mar 18.

Zid, et al., 2009 Zid, Brian M; Rogers, Aric N; Katewa, Subhash D; Vargas, Misha A; Kolipinski, Marysia C; Lu, Tony Au; Benzer, Seymour; & Kapahi, Pankaj (2009). '**4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila.**' *Cell*. 139(1), pp. 149-60.

Zimmerman, et al., 2003 Zimmerman, Jay A; Malloy, Virginia; Krajcik, Rozlyn; & Orentreich, Norman (2003). '**Nutritional control of aging.**' *Experimental gerontology*. 38(1-2), pp. 47-52.

Zornetzer, et al., 1982 Zornetzer, S F; Thompson, R; Rogers, J (1982). **"Rapid forgetting in aged rats."** Behav Neural Biol. 1982 Sep;36(1):49-60.

Zou, et al., 2016 Zou, Shitao; Shang, Zeng-Fu; Liu, Biao; Zhang, Shuyu; Wu, Jinchang; Huang, Min; Ding, Wei-Qun; Zhou, Jundong (2016). **"DNA polymerase iota (Pol iota) promotes invasion and metastasis of esophageal squamous cell carcinoma."** Oncotarget. 2016 May 31;7(22):32274-85. doi: 10.18632/oncotarget.8580.